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THE ROLE OF PROSTAGLANDINS IN HUMAN GALLBLADDER MOTILITY

by

© CYRUS KOTWALL

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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DEDICATED

TO

MY WIFE

CAROL

ABSTRACT

This study investigated the role of prostaglandins in the control of human gallbladder motility.

Fresh human gallbladders obtained at cholecystectomy underwent pharmacological studies in organ baths to determine the effects of exogenous prostaglandins on gallbladder motility. For comparative purposes prostaglandin-induced motility changes in the guinea-pig gallbladder were also studied. To relate motility studies in the human gallbladder with prostaglandin release, reverse-phase high performance liquid chromatography was used. All human gallbladders, according to the severity of cholecystitis, were classified by a histological scoring system as chronic mild, chronic advanced or acute cholecystitis.

Spontaneous activity in the human muscle strips was found to vary amongst the different disease groups and was due in whole or in part to endogenous prostaglandin production. Prostaglandins of the E series were the most likely prostaglandins involved in regulation of endogenous tone in the human gallbladder.

PGE_1 and PGE_2 were the most potent prostaglandins inducing contractile responses in the human gallbladder with chronic mild and advanced cholecystitis. Prostacyclin induced no effects in human gallbladder motility but was fairly potent in inducing contractile responses in guinea-pig gallbladder. Leukotrienes were without effect in human gallbladder motility but were very potent contractile agents in guinea-pig gallbladder. No prostaglandin-induced responses were found

in gallbladders with acute cholecystitis and this may be due to increased levels of endogenous prostaglandins in these tissues.

The main prostaglandin spontaneously released from human gallbladder tissue was PGE_2. Calcium ionophore-stimulated prostaglandin release from human tissue revealed that PGD_2 was the predominant prostaglandin released. Leukotrienes were also released from human tissue in both the spontaneous and stimulated states.

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INTRODUCTION

PROSTAGLANDINS AND LEUKOTRIENES

History of prostaglandins

Prostaglandin literature extends back to the 1930s when von Euler [1] and Goldblatt [2] independently observed very specific properties of human seminal fluid. Von Euler, a Swedish physiologist, determined that extracts from human prostate and seminal vesicles, and sheep vesicular glands, caused vasodilation and increased intestinal and uterine activity, actions which are still true today. The word "prostaglandin" comes from von Euler's work in 1935 [1], where he erroneously concluded that these substances originated from the prostate gland.

In 1962, prostaglandin E_1(PGE_1) and $PGF_{1\alpha}$ were isolated by Bergström [3], and it was these initial steps that led to the important discovery by Vane that aspirin produces its effects by synthesis inhibition of prostaglandins[4]. Noting that the lungs of guinea-pigs when challenged released prostaglandins, he found that this release was antagonized by aspirin-like drugs. This was further substantiated by Ferreira, Moncada and Vane when they stimulated dog spleen to produce large amounts of prostaglandins[5]. They similarly found that indomethacin and aspirin inhibited prostaglandin release. These discoveries generated a tremendous amount of research into the effects of prostaglandins on all organ systems, as aspirin is one of the most widely used therapeutic agents.

Hamberg and Samuelsson in 1973 [6] identified two unstable cyclic endoperoxides, prostaglandin G_2 and PGH_2 which are key intermediates in

prostaglandin biosynthesis. This was followed shortly by the isolation of the thromboxanes in 1975[7]. Moncada et al. [8,9] in 1976 found a substance from arterial walls that potently inhibited platelet aggregation. This was called prostaglandin X, and was subsequently renamed prostacyclin, a drug of particular importance in vascular homeostasis. In 1979, Samuelsson et al. [10] discovered an entirely new group of metabolites of fatty acids, which were later named leukotrienes. This considerable effort into prostaglandin research was ultimately recognized when the 1982 Nobel Prize for medicine was jointly awarded to Vane, Bergstrom and Samuelsson.

Biochemistry of Prostaglandins and Leukotrienes

Fig. 1 depicts four main metabolites of arachidonic acid - prostaglandins, thromboxanes, prostacyclin and the leukotrienes. These related compounds are derived from 20-carbon unsaturated essential fatty acids containing three, four or five double bonds: 8, 11, 14 - eicosatrienoic acid (dihomo-gamma-linolenic acid), 5, 8, 11, 14 - eicosatetraenoic acid (arachidonic acid) and 5, 8, 11, 14, 17 -eicosapen taenoic acid. These fatty acid precursors give rise to various prostaglandins denoted by subscripts 1, 2 or 3, respectively, with arachidonic acid being the most abundant precursor in man. Therefore, prostaglandins of the 2 series assume the most importance.

Arachidonate is derived from dietary linoleic acid or directly from ingested meat, and then esterified as a component of most cell membrane phospholipids. Arachidonate cannot be utilized in prostaglandin synthesis unless it is released from the cell membrane. Phospholipase A_2 is the major enzyme cleaving arachidonate from phospholipids

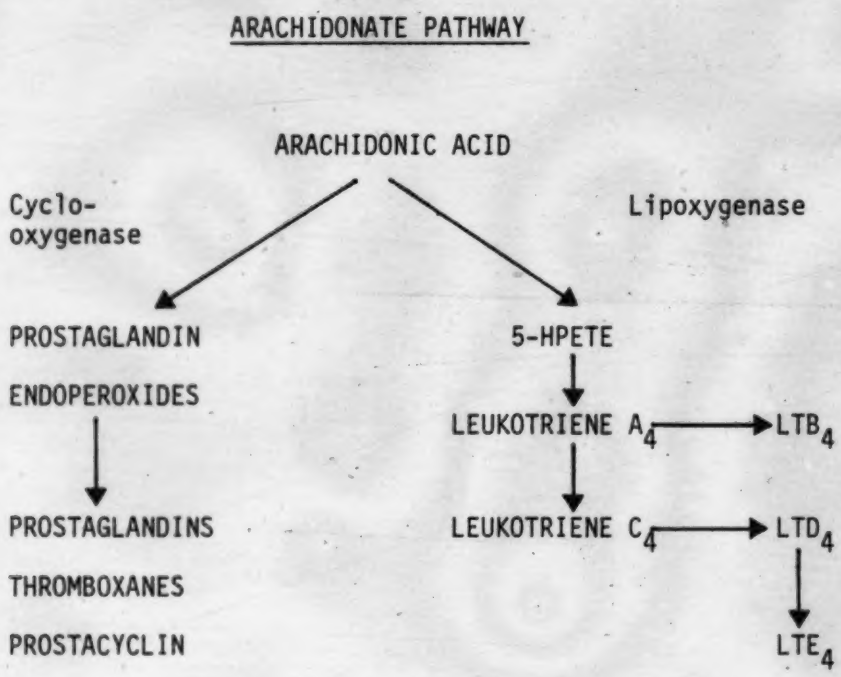


FIGURE 1

Schematic Diagram Illustrating
the Arachidonic Acid Cascade

including phosphatidylcholine, phosphatidylinositol, phosphatidylserine and phosphatidylethanolamine. Phospholipase C may also participate in arachidonate release[11]. Free arachidonate is then available to the cell microsomes and the cell membrane where two critical enzymes, a cyclooxygenase and a lipoxygenase rapidly metabolize arachidonic acid to its oxygenated products.

All mammalian cells appear to contain these necessary enzymes, and so possess the ability to produce prostaglandins. Prostaglandins are not stored in the cell and therefore production reflects immediate biosynthesis. The level of arachidonate is very low in the cell, and it is believed that the rate of prostaglandin synthesis depends upon phospholipase-induced release of arachidonate from membrane phospholipids[12]. Moreover, phospholipase activation may be the rate limiting step in increasing prostaglandin biosynthesis in response to physical, chemical and hormonal stimuli[13,14].

Cyclooxygenase Pathway

An enzyme complex known as "prostaglandin synthetase" is composed of two distinct enzymes, a cyclooxygenase and a lipoxygenase[15]. Cyclooxygenase adds oxygen to arachidonate and cyclizes it to the cyclic endoperoxide PGG_2. This is converted to PGH_2 by the peroxidase activity of the enzyme, with the production of toxic unstable oxygen radicals[16]. These endoperoxides are unstable with half lives of about 5 min in physiological media and are enzymatically converted to one or more of the "classic prostaglandins" - PGE_2, $PGF_{2\alpha}$ and PGD_2. PGA, PGB and PGC arise during chemical extraction of PGE; probably none occur biologically[12]. All six prostaglandins have a cyclopentane ring, and

the letter designations reflect different functional groups attached to the ring.

In addition to prostaglandins, PGH_2 is metabolized to two other products. Thromboxane synthetase forms thromboxane A_2 which is very unstable possessing a half-life of 30 s, and is degraded to the stable, but biologically inactive thromboxane B_2. Similarly, prostacyclin synthetase converts PGH_2 to prostacyclin (PGI_2) which is also unstable with a half-life of 3 min. 6-keto-$PGF_{1\alpha}$ is the stable degradation product of prostacyclin and is 1000 times less active.

Enzymes catalyzing prostaglandin degradation are widely distributed in body tissues, and inactivation occurs relatively quickly. Ferreira [17] found that 95% of infused PGE_2 is inactivated during one passage through the pulmonary circulation, and Hamberg [18] showed that only 3% of an intravenous dose of PGE_2 remained in the plasma after 90 s. It appears that the lungs are the major site of prostaglandin inactivation, but in perfused lungs *in vitro* and *in vivo*, PGA and PGI_2 pass through the pulmonary circulation without loss of activity[14].

All tissues can synthesize the intermediate endoperoxides, but the distribution of further products depends upon the tissue. Most tissues cannot produce the entire range of compounds, except for the lung and spleen; platelets and blood vessel wall produce mainly PGI_2 and TXA_2, respectively.

Lipoxygenase Pathway

Lipoxygenases have only been found in the lung, platelets and leukocytes, in contrast to the cyclooxygenase which is widely distributed[12]. The action of lipoxygenase on arachidonate yields 5-

hydroperoxyeicosatetranoic acid (5-HPETE) and hydroxyeicosatetranoic acids (HETEs). 5-HPETE is the biochemical precourser of leukotriene A_4 (LTA_4), as illustrated in Fig. 1.

Leukotrienes are noncyclized 20-carbon carboxylic acids containing three conjugated double bonds. The word "leukotriene" originates from their discovery in rabbit leukocytes[10,20], and their conjugated triene unit. The subscript "4" indicates the total number of double bonds. Leukotrienes are important mediators of immediate hypersensitivity, and it is now established that LTC_4 and LTD_4 are the critical components of slow reacting substance of anaphylaxis (SRS-A)[21-23].

Inhibitors of the Arachidonate Pathway

Drugs inhibiting the arachidonate cascade may be classified into four general groups.

1. inhibitors of phospholipase A_2 and thereby arachidonate release (eg. corticosteroids).
2. inhibitors of cyclooxygenase enzyme, thereby blocking synthesis of prostaglandins, thromboxanes and prostacyclin (eg. aspirin and indomethacin).
3. inhibitors of the lipoxygenase pathway (eg. nordihydroguaiaretic acid).
4. more specific inhibitors of final products (imidazole).

Corticosteroids possess numerous actions all of which could contribute to their anti-inflammatory effects. These include maintenance of the micro-circulation with decreased plasma exudation;

cell membrane stability with lysosomal stabilization; inhibition of leukocyte chemotaxis, adhesion, phagocytosis and bactericidal capacity; and T-lymphocyte function inhibition[24]. In addition, steroids have been documented to limit availability of the substrate arachidonic acid for prostaglandin biosynthesis [25], and only recently has the exact mechanism of inhibition been elucidated. Flower and Blackwell [26] have suggested that steroids stimulate production of a protein which inhibits phospholipase A_2, and recently have identified a polypeptide causing an anti-phospholipase effect and named it "macrocortin"[27]. They believe this to be a second messenger mediating the antiphospholipase effect of steroids.

One further effect of corticosteroids should be mentioned. In prostaglandin biosynthesis[16] and during inflammation, phagocytosis and leukocyte activation, toxic oxygen moieties are released[28]. These toxic radicals have harmful cellular effects including DNA denaturation, enzyme inactivation and formation of toxic lipid peroxides[28]. These effects may well be involved in the inflammatory response, and steroidal drugs have the ability to scavenge oxygen radicals[29,30]. In general, therapeutic efficacy of the anti-inflammatory drugs, parallels their ability to block both the cyclooxygenase and lipoxygenase enzymes, and therefore corticosteroids are considered one of the most potent anti-inflammatory agents.

Indomethacin and aspirin are selective cyclooxygenase enzyme inhibitors. Indomethacin reversibly interacts with the substrate binding site of cyclooxygenase, whereas aspirin irreversibly inhibits the enzyme by acetylating its amino-terminal serine group. In the rabbit leukocyte, indomethacin has been shown to inhibit phospholipase

A_2[31]. Aspirin has a very potent action in platelets compared to other tissues, with 20 mg/day markedly inhibiting thromboxane synthesis and thereby platelet aggregation [32,33]. Similar to corticosteroids, both indomethacin [30,34] and aspirin [35] are oxygen radical scavengers. Although neither drug inhibits the lipoxygenase, indomethacin and aspirin both inhibit the conversion of HPETE to HETE [36]. Since HETE is chemotactic for polymorphonuclear leukocytes [37], and HPETE has an inhibitory effect on prostaglandin synthesis [36], this provides a further anti-inflammatory effect.

Turning to the lipoxygenase enzyme, nordihydroguaiaretic acid (NGA) is a selective inhibitor of the lipoxygenase pathway [38]. NGA therefore blocks synthesis of both HPETE and HETE, and leukotrienes. 1-phenyl-3-pyrazolidone (phenidone) is effective against both the cyclo-oxygenase and lipoxygenase pathways [39], but is less effective than NGA in lipoxygenase inhibition [38]. Augstein [40] developed a very specific and potent antagonist of SRS-A called FPL55712, and this has been confirmed in later studies[41,42]. Imidazole is the only selective inhibitor on the cyclooxygenase side, inhibiting thromboxane synthetase. These latter drugs are not used clinically, but are of importance as pharmacologic tools in the laboratory.

THE INFLAMMATORY RESPONSE

There is a wealth of literature implicating prostaglandins and leukotrienes in the inflammatory and hypersensitivity responses,

respectively. Histamine and bradykinin are two well known inflammatory mediators. In general, it can be said that the products of arachidonic acid greatly potentiate the effects of histamine and bradykinin in the inflammatory response.

Contribution by Prostaglandins

Drugs which are potent anti-inflammatory agents only weakly inhibit histamine, serotonin and bradykinin, whereas drugs that are effective against these agents are poor anti-inflammatory agents. This fact alone suggests a major role for prostaglandins as pro-inflammatory agents.

If one reviews the five cardinal signs of inflammation - rubor, tumor, calore, dolore and functio laesa, one can associate prostaglandins with each of these signs. Firstly, erythema is due to vasodilation; PGE, PGA, PGD and prostacyclin are vasodilators, in contrast to the thromboxanes and PGF which are vasoconstrictors. Moreover, PGE causes long-lasting erythema and exacerbates edema and pain induced by bradykinin and histamine[43,44]. Secondly, prostaglandins by themselves are not potent inducers of edema and have little action on vessel permeability. However, PGE intensified rat paw edema induced by histamine, bradykinin or carrageenin[45], and Williams feels that prostaglandins sensitize blood vessels to the permeability effects of other mediators to increase exudation[46]. Thirdly, PGE is a very potent mediator in the febrile response. PGE is the most powerful pyretic agent known when injected into the cerebral ventricles of cats[47]. Saxena et al.[48] found increased PGE-like activity in the cerebrospinal fluid (CSF) of patients with high fever and bacterial or viral sepsis; his afebrile control group had no PGE activity in the

CSF. Also, fever is a frequent side-effect of intravenous $PGF_{2}\alpha$ when given as an abortifacient[49].

Fourthly, in concentrations found at inflammed sites, prostaglandins do not cause overt pain. Prostaglandins administered to mice induced writhing responses[50]; in rat paws, PGE_2 produced a marked decrease in pain threshold [51]; and in man, prostaglandins cause intense pain when injected intradermally on the volar surface of the arm[52]. A major feature is the production of a hyperalgesic state by prostaglandins[52], and the sensitization of pain receptors to bradykinin, histamine[53], and any chemical or mechanical stimulation[51,52]. Lastly, loss of function (functio laesa) implies cellular involvement with infiltration of platelets, leukocytes, macrophages and lymphocytes. PGE_2 potentiates platelet aggregation induced by ADP[54]. PGD_2 and PGE_2 enhance leukocyte chemotactic activity of LTB_4 in monkey skin[55], and it is well known that HETE is chemotactic for polymorphonuclear leukocytes[37,52]. Moreover, leukocytes that have arrived at the inflammatory site within six hours are a major source of TXB_2, PGE_2 and 6-oxo-$PGF_{1}\alpha$ (a degradation product of PGI_2), which serve to heighten the inflammatory response[56]. Macrophages, in addition produce prostacyclin as well as PGE_2[57].

Many forms of inflammation with tissue damage reveal increased quantities of prostaglandins, especially PGE. These include thermal injury to guinea-pig skin[58], anaphylaxis[59] and rheumatoid arthritis[60]. Although the evidence for significant prostaglandin involvement in the inflammatory response and tissue damage is convincing there are opposing views. Brune [61] feels that prostaglandins are not the most important mediators of inflammation; it is also known that

prostaglandins in high enough doses will suppress rheumatoid inflammation, lymphocyte or antibody-mediated cytotoxicity and leukocyte migration[62,63].

Summarizing the contribution by prostaglandins in the inflammatory response, one can say that prostaglandins are involved and play a major role in enhancing the response to histamine and bradykinin. Prostaglandins are very potent local mediators that are released by a variety of stimuli. These include mechanical (eg. stretch)[64], thermal, chemical and bacterial[51,52] stimuli.

Contribution by Leukotrienes

Lipoxygenase products are also involved in the inflammatory response and assume even more importance as mediators of immediate hypersensitivity. As mentioned previously, HETE is a potent leukocyte chemotactic agent, and LTB_4 exhibits equipotent chemotactic activity to one of the complement proteins, C_5a[65,66]. Leukotrienes exert a synergistic action with PGE_2 and PGD_2 with an augmented cutaneous infiltrate elicited by all three compounds when injected into rabbit, monkey and human skin[67]. Biopsy of an intradermal injection of LTB_4 and PGD_2 in man, reveals numerous neutrophils[68], again confirming chemotaxis.

Leukotrienes are also very potent bronchoconstrictive agents, which is an important factor in hypersensitivity. LTC_4 and LTD_4 are 200 and 20,000 times more potent than histamine, respectively, in contracting strips of guinea-pig lung[69], and in man LTC_4 is bronchoconstrictive[70]. LTB_4 increases vascular permeability in human skin[68] and when combined with LTD_4 and PGE_2, there is an enhancement

of cutaneous vascular permeability in various animals[67].

Therefore, leukotrienes are much more potent mediators of hypersensitivity than histamine. They also demonstrate an additive effect with prostaglandins in the inflammatory response.

PHYSIOLOGIC ACTIONS OF PROSTAGLANDINS

The literature related to the actions of prostaglandins is vast. Arachidonate metabolites are rapidly inactivated in the circulation by the lungs and the liver. They are thus sensitive local intracellular modulators of biochemical activity; since most cells command the necessary machinery to synthesize prostaglandins, it is natural that they have effects on most organ systems. In particular, prostaglandins have powerful effects on the cardiovascular, respiratory, gastrointestinal, female reproductive and urinary system. Recently, they have also been found to play a role in oncology and immunology.

Vasoactive prostaglandins include PGE, PGA and PGI_2 which are vasodilators and $PGF_{2\alpha}$ and TXA_2 which are vasoconstrictors. These agents act directly on the smooth muscle of the vessel wall. Intra-arterial PGE_1 has been used in the treatment of ischemic peripheral vascular disease[71]. Occlusion of coronary vessels induces synthesis of PGE_2 and PGI_2, and indomethacin has been shown to inhibit the vasodilatory response to hypoxemia[72]. Prostaglandins may regulate blood vessel tone as it is postulated that in essential hypertension there is a deficiency in renal production of PGE_2[73]. PGE_2 is a ductus arteriosus dilator and indomethacin is currently used in effecting

ductal closure in neonates[74].

The role of arachidonate metabolites and the platelet is essential in hemostasis. Prostacyclin, synthesized by endothelial cells is a potent inhibitor of platelet aggregation[75]. Thromboxane A_2, a powerful platelet aggregator, is hence in critical balance with PGI_2 in control of blood flow. Prostacyclin stimulates adenylate cyclase, leading to increased cyclic 3', 5' adenosine monophosphate (cAMP) in the platelet[76] which correlates well with its antiaggregating activity compared with PGD_2 and PGE_1, as PGI_2 is 10 times more active than PGD_2 and 30 times more active than PGE_1[77]. Prostacyclin shows promise in cardiopulmonary bypass where it reduces platelet deposition on the filter mesh, and so reduces postoperative thrombocytopenia[78]. Similar to release of prostaglandins by chemical and mechanical stimuli[51,52,64], Ritter has demonstrated prostacyclin release from human blood vessels <u>in vivo</u> following distension of forearm veins with saline[79].

As previously mentioned, leukotrienes are potent bronchoconstrictors, an action also shared by $PGF_{2\alpha}$ and TXA_2. In contrast, PGE_2 and PGI_2 are bronchodilators, and an imbalance between $PGF_{2\alpha}$ and PGE_2 may contribute to a high bronchial tone in asthmatics[80]. Also, in keeping with prostaglandin involvement in the inflammatory response, increased levels of PGF have been demonstrated following allergen-induced asthma[81].

Increased uterine activity was one of the first actions of prostaglandins elucidated by von Euler, and the human uterus <u>in vivo</u> is always contracted by PGE and $PGF_{2\alpha}$. These prostaglandins are presently used as abortifacients. That prostaglandins contribute to uterine tone

is also demonstrated by Lewis' findings that pregnant women ingesting high doses of aspirin had a significant increase in the length of gestation and duration of labour[82]. In addition, increased prostaglandin levels may exist in dysmenorrheic women[83].

Infusion of prostaglandins of the E series, PGA and PGI_2 into canine renal arteries increases renal blood flow with diuresis, natriuresis and kaliuresis[84]. Indeed furosemide (Lasix) induces diuresis, by stimulating prostaglandin synthesis[85]. Bartters syndrome, manifested by elevated plasma renin and hypokalemia, is due to increased prostaglandin formation and clinically responds to indomethacin[86].

Prostaglandins suppress immune responsiveness, and inhibit T and B-lymphocyte functions[87]. They also inhibit lymphokine production by sensitizing T-cells[88]. Of even more importance is their involvement with malignancy. In general, prostaglandins exert inhibitory actions on tumour cell proliferation. Bennett [89] has demonstrated increased prostaglandins in human lung carcinomas; he has also shown that survival is inversely related to prostaglandin content from human breast cancers[90]. Additionally, PGE_2 is a potent inducer of bone resorption, and the hypercalcemia associated with some neoplasms may be due to increased levels of PGE_2[91].

From this overview, one can state that prostaglandins are ubiquitous, possess multiple and diverse actions, and are very potent local mediators especially in the inflammatory response. We shall now examine prostaglandins in relation to the gastrointestinal tract, and then more specifically in reference to the human gallbladder.

PROSTAGLANDINS AND THE GASTROINTESTINAL TRACT

Prostaglandins produce distinct effects in the entire gastrointestinal tract. In the stomach, they exert potent anti-secretory and cytoprotective actions; in the small bowel, diarrhea is induced and in the colon, prostaglandins may mediate the effects of inflammatory bowel disease.

Stomach

In vivo and *in vitro* animal and human studies have established that prostaglandins of the E series, PGA and prostacyclin are anti-secretory. Synthetic methyl analogues are potent inhibitors of gastric acid secretion, and the mechanism of action includes both a decrease in gastric mucosal blood flow and an inhibitory action on the ability of histamine to stimulate cAMP. Of even more interest is the cytoprotective nature of prostaglandins when the gastric mucosa is exposed to noxious agents or non-steroidal anti-inflammatory compounds. This is a different mechanism of action, as the doses required are far too small to inhibit gastric secretion[92]. Robert has found that pretreatment of the gastric mucosa by "mild" irritants followed by necrotizing agents, prevents mucosal damage by stimulating formation of endogenous cytoprotective prostaglandins[93]; he has called this "adaptive cytoprotection". Cytoprotection may be mediated by enhanced mucous secretion, effects on the sodium pump or chloride transport and increased cAMP concentration[94]; recently, Himal [95] has demonstrated that PGE_2 prevents acute gastric erosions by stabilizing lysosomal membranes. This has obvious clinical implications in

accelerating duodenal ulcer healing[96], preventing aspirin damage to gastric mucosa[97] and aspirin-induced blood loss in man[98].

Small Bowel

Prostaglandins produce diarrhea in the small intestine mediated by either (or both) hypermotility or secretion of fluid into the lumen. Prostaglandins of the E and F series, PGA, and prostacyclin stimulate the longitudinal smooth muscle of the gut, whereas prostaglandins of the E series relax the circular smooth muscle and prostaglandins of the F series contract both muscle layers[94]. This was also confirmed by Bennett et al.[99] as aspirin and indomethacin inhibited peristaltic activity in guinea-pig ileum and colon. $PGF_{2\alpha}$, the most potent prostaglandin in effecting intestinal smooth muscle contraction[94], has been proposed as a possible treatment of paralytic ileus[100].

Robert [101] believes that the diarrhea induced by exogenous prostaglandins is due to intestinal fluid secretion and not hypermotility; he has called this "enteropooling". Similar to mechanical and chemical stimuli causing release of prostaglandins[51,52,64], distension-induced secretion has been documented in the rat ileum[102]. PGE_1, PGE_2 and $PGF_{2\alpha}$ are enteropooling, whereas prostacyclin and PGD_2 are anti-enteropooling and will block a potent enteropooling agent, cholera toxin[103]. The mechanism of action includes both an inhibition of glucose and water absorption[104], and also sodium and chloride absorption in human jejunum[104,105] and in rabbit ileum[106]. Biochemically, prostaglandins induce diarrhea by stimulating production of cAMP in small intestine[107]. Although prostaglandin-induced diarrhea resembles

that produced by cholera toxin and vasoactive intestinal polypeptide (VIP)[94], cholera toxin-induced diarrhea is not due to elevated cAMP[107,108]. In accordance with the action of prostacyclin being anti-entropooling, it is a very weak stimulator of cAMP[109]. Clinically, the diarrhea associated with medullary carcinoma of the thyroid, carcinoid tumours and the watery diarrhea-hypokalemia achlorhydria syndrome (WDHA) has been linked to hyperprostaglandinemia[110].

Colon - Inflammatory Bowel Disease

Since prostaglandins have pro-inflammatory actions and induce diarrhea, many investigators have tried to implicate prostaglandins with inflammatory bowel disease (IBD). Gould [111] was one of the first to provide evidence for increased levels of prostaglandins in active ulcerative colitis, and this was confirmed in three subsequent studies[112-114]. However, all these studies raise the question of whether the inflammation was caused by elevated prostaglandins, or that the inflammation secondarily induced high prostaglandin levels.

Sulfasalazine (salicylazosulfapyridine) is widely used in the treatment of IBD. After oral ingestion, most of the drug reaches the colon intact and is split by bacterial enzymes into sulfapyridine and 5-aminosalicyclic acid (5-ASA). It is believed by some that 5-ASA is the active therapeutic moiety due to its inhibitory action on prostaglandin synthesis, thereby removing excess pro-inflammatory prostaglandins [115-117]. However, Hoult and Moore [118,119] demonstrated that Sulfasalazine is a potent inhibitor of prostaglandin 15-hydroxydehydrogenase; this enzyme catalyzes the first step in PGE and

PGF inactivation and would thereby potentiate the biologic actions of prostaglandins. They felt this may be beneficial during the stage when mucosal erosion would be precipitated by prostaglandin deficiency. Hoult [120] also showed that 5-ASA increased prostacyclin formation and would therefore again increase prostaglandin levels. This dual effect of Sulfasalazine on prostaglandin synthesis is possibly explained by Schlenker [121] as being dependent on either a high or low concentration of substrate (arachidonate) in human colonic mucosa. It is clear more work is needed to determine the therapeutic effectiveness of Sulfasalazine in either decreasing or increasing prostaglandin levels in ulcerative colitis.

There is also evidence for increased prostaglandin levels in Crohn's disease[122,123] and Rachmilewitz [123] feels that the increased synthesis is from intestinal inflammatory mononuclear cells. In summary, there are high levels of prostaglandins in IBD, but it is not known whether this is a cause or an effect. Also the exact mechanism of action of Sulfasalazine is not yet known. Since we know that prostaglandins in the stomach are cytoprotective, it is tempting to say that the same action holds true in the colon and that Sulfasalazine increases cytoprotective prostaglandins, but at the present time evidence is lacking.

THE HUMAN GALLBLADDER

Approximately 20 million people in the United States alone have disease of the biliary tract and about 500,000 cholecystectomies are

performed annually. Gallstones comprise by far the major portion of biliary tract disease, and it is easy to justify the tremendous amount of research into cholelithiasis and its attendant complications. The incidence of cholelithiasis increases with age; in persons over 40 years of age about 20 percent are affected, thus making cholelithiasis one of the most common diseases of adult life.

Anatomy

The gallbladder, cystic duct and common bile duct develop from the caudal portion of a foregut diverticulum, whereas the liver and intrahepatic bile ducts develop from the cranial portion. The gallbladder is a thin-walled organ attached to the inferior surface of the junction between the right and left lobes of the liver. In the absence of disease it has a capacity of 30 to 60 ml. The gallbladder is divided into a fundus, body, infundibulum and neck from proximal to distal. The neck is in continuity with the cystic duct, which is about 2 to 4 cm long, and contains mucosal folds called the valves of Heister. The cystic duct joins the common hepatic duct to form the common bile duct which enters the duodenum at the papilla of Vater. The choledochoduodenal junction is formed by a specific complex of four muscles known collectively as the sphincter of Oddi.

Histologically, the gallbladder consists of a mucosa, muscularis and serosa. The epithelium is columnar and mucus glands are found only in the neck. In contrast to the rest of the gut, which is composed of three muscle layers - muscularis mucosa, inner circular and outer longitudinal - the gallbladder has only one thin layer of muscle resembling that of the muscularis mucosa. This layer in the dog is a

three-dimensional mesh of random interconnecting muscle bundles [124], and probably the human muscle layer is similar.

The major stimulatory innervation of the gallbladder is parasympathetic by way of the anterior hepatic branch of the vagus nerve. Sympathetic and a non-adrenergic inhibitory innervation also exist, but the role of the latter in gallbladder motility is presently not clear.

Normal Gallbladder Motility and Absorption

Bile is composed of a mixture of bile salts, bile pigments, cholesterol, lecithin, inorganic electrolytes, fatty acids and protein, water and products of hepatic metabolism. The bile salts are glycine and taurine conjugates of two primary bile acids, cholic and chenodeoxycholic acid, which are synthesized from cholesterol in the liver. There are two secondary bile acids, deoxycholic and lithocholic acid formed in the gut by bacterial action on the primary acids; all are reabsorbed in the terminal ileum except lithocholic acid and resecreted in an enterohepatic circulation. The rate of return to the liver of bile acids determines the rate of hepatic synthesis.

The liver secretes 600 to 1000 ml of bile per day and the tremendous absorptive capacity of the gallbladder concentrates the bile at least 5 to 10 times. During fasting, only 50% of bile secreted by the liver enters the gallbladder; the remainder directly enters the duodenum[125]. Bile gains entry into the gallbladder due to its low resting pressure, about 10 cm of water, compared to the biliary secretory pressure in the liver of 30 cm of water[126]. Once inside the gallbladder, concentration of bile is initiated by absorption of NaCl

and $NaHCO_3$ with water following by osmotic coupling; this produces a serosa-positive diffusion potential. This is an active process and requires NaK-ATPase. The main barrier regulating gallbladder permeability is the epithelium where a NaCl co-transport mechanism is located[127]; this is very selective for Na^+ but absorbs HCO_3^- and other anions in place of Cl^-. Absorbed fluid passes through the lateral intercellular space with the driving force being hypertonic NaCl, and fluid is prevented from flowing back into the lumen by tight junctions[127]. This model is compatible with the initial classic studies on gallbladder fluid absorption by Diamond[128].

The main stimulus for gallbladder contraction is hormonal as opposed to neural via the vagus nerve. Cholecystokinin (CCK) is the most important hormone in this regard, and is located in the "I" cell of the small bowel[129]. CCK is released upon entry of essential amino acids (especially phenylalanine and tryptophan), fatty acids and hydrogen ions into the duodenum[130]. CCK acts directly on smooth muscle to effect gallbladder contraction, sphincter of Oddi relaxation and increased duodenal motility. Biochemically, CCK decreases intracellular cAMP[131], and may also increase the levels of cGMP[132]. CCK is also a choleretic, increasing the flow of bile. Another gut hormone, secretin, potentiates the actions of CCK on gallbladder contraction and choleresis.

The cystic duct has been neglected for years as a participant in biliary motility, but Lennon has established that the cystic duct plays an important role in bile-flow dynamics[133]. In summary, biliary motility in the absence of disease is regulated by hormonal, neural and structural factors.

The Pathophysiology of Gallstones

The literature regarding the pathophysiology of gallstones is extensive. Descriptions of biliary calculi date back to 1000 B.C. in the Nile Valley, and cholesterol was identified as the major component of gallstones some 200 years ago; it is surprising that the pathogenesis of gallstones is still not completely understood.

There are three major types of calculi, with two of these types containing cholesterol as the major component and together constituting 90% of stones. Mixed cholesterol stones account for 80%, and the remainder of the calculi are equally divided between pure cholesterol and pigment stones. Thus cholesterol is the predominant component of biliary calculi. The initial focus on pathogenesis of gallstones revolved around infection in the gallbladder wall leading to exfoliation of inflammatory cells that served as a nidus for stone formation, and later around gallbladder stasis that favoured cholesterol precipitation. Only recently is it known that the most important factor in stone formation is the physicochemical composition of bile.

Bile is composed of three main constituents - bile salts, lecithin and cholesterol. In contrast to bile salts, the last two are water insoluble, but become soluble when incorporated into a bile salt-lecithin-cholesterol micelle. Admirand and Small [134] were one of the first to determine that a relative increase in cholesterol or a decrease in lecithin or bile salts leads to a state of cholesterol supersaturation. Small [135] also showed that the liver is the source of lithogenic bile.

There are three factors involved in the secretion of supersaturated

bile. First, increased secretion of cholesterol has been demonstrated in obese patients with excessive cholesterol synthesis[136]. Second, decreased secretion of bile salts is well established in disease or resection of the terminal ileum. Thirdly, in the majority of gallstone-formers, subtle alterations occur in the enterohepatic circulation of bile salts leading to a relative increase in cholesterol secretion [137-139]. It is also known that during fasting, there is an interruption of the enterohepatic circulation with production of supersaturated bile[140]. This firmly establishes that supersaturated bile is a prerequisite for cholelithiasis, but many individuals have supersaturated bile with no gallstones[140,141]. This means that there is some other factor or factors in addition to cholesterol supersaturation that is necessary for cholesterol cholelithiasis.

Once cholesterol nucleation and crystallization occur, stone formation is inevitable[142]. However, the step between supersaturation and crystallization is large and other factors such as mucus secretion, changes in fluid transport and stasis may be important.

Pathogenesis of Acute Cholecystitis

Acute cholecystitis is associated with gallstones in 95% of cases, and this is one of the factors responsible in the pathogenesis of cholecystitis. The initiating event is impaction of a stone in the gallbladder neck, thereby obstructing the cystic duct. Ischemia then develops secondary to gallbladder distension. This results in a "chemical" cholecystitis as this is clearly an inflammatory injury and bacteria cannot be found in significant amounts. Only after this initial chemical injury a secondary bacterial cholecystitis develops

with a 60% incidence of positive bacterial cultures.

It is the chemical injury that is the most interesting. Anderson [143] postulated injury secondary to pancreatic juice, but the most appealing studies concern Sjödahl's work with lysolecithin[144]. Lysosomes contain phospholipase A which converts lecithin, a normal bile constituent, to lysolecithin. This induces structural changes in biologic membranes due to its surface active properties and thereby cell damage with an associated inflammatory response; this has been shown in the rabbit gallbladder[145]. Moreover, Sjödahl [144] has shown an elevated lysolecithin to lecithin ratio in bile from patients with acute cholecystitis. He postulates that the impacted stone causes epithelial damage with release of phospholipase A from lysosomes, leading to an increase in the potent inflammatory mediator lysolecithin. In addition to lysolecithin, bile salts and cholesterol-saturated bile when combined with cystic duct occlusion, are irritants and induce acute cholecystitis[146,147].

Hence, the literature points towards lysolecithin as the main mediator of chemical injury. Lysophosphatidylcholine, another phospholipid in gallbladder bile, has also been shown to exert potent cytotoxic properties on guinea-pig gallbladder[148]. Neiderhiser et al. [149] have found that lysophosphatidylcholine in the cat induces secretion into the lumen and contraction of the gallbladder; moreover, indomethacin abolished these actions indicating they were mediated by prostaglandins. They did not mention an inhibition of the cytotoxic effect by indomethacin but it seems plausible that prostaglandins could be released from the gallbladder wall by mechanical and chemical stimuli; from our knowledge of their potent pro-inflammatory and

hyperalgesic actions they may well potentiate the initial chemical and subsequent bacterial cholecystitis. We will now look more closely at the evidence implicating prostaglandins in gallbladder function and disease.

PROSTAGLANDINS AND THE GALLBLADDER

Mucus Secretion

The finding that individuals without gallstones secrete cholesterol-saturated bile indicates that additional factors are necessary for the production of cholesterol stones[140,141]. These other factors include mucus, enhanced fluid transport across the gallbladder wall[150], and stasis such as in pregnancy or in patients with diabetes mellitus. Of these, we shall discuss mucus secretion.

Mucins are very high molecular weight glycoproteins secreted by goblet cells of many epithelial-lined organs. Gallbladder epithelium is devoid of these cells, but all the surface cells have secretory granules containing mucin glycoproteins. As early as 1963 Womack suggested the importance of mucus[151], and in a later study in hamsters stated that a prosthesis of mucus was essential for the development of gallstones[152]. Subsequently, Lee and LaMont [153] found that in cholesterol-fed prairie dogs there was mucus hypersecretion, and that the mucous served as a nucleating agent for cholesterol. The cholesterol induced histologic changes with increased mucous granule size and number in the cytoplasm of the mucosal cells[154]. Furthermore, aspirin given to cholesterol-fed prairie dogs inhibited

mucin hypersecretion and prevented gallstone formation[155]; aspirin was chosen as it is known to inhibit gastric mucous production[156]. Recently, LaMont [157] found that incubation of arachidonate with prairie dog gallbladder epithelium significantly stimulated mucin release, and moreover this release was inhibited by indomethacin. These findings provide strong evidence that endogenous prostaglandin synthesis is an important regulator of mucin release, and consequently also in gallstone formation.

Fluid Transport

One of the main functions of the gallbladder in man and several species is to concentrate bile. This was recognized over 60 years ago by Rous and McMaster[158], but it is only recently that prostaglandins have been shown to be involved in fluid transport. The classic study by Diamond in 1962 [128] in the fish gallbladder demonstrated absorption of isotonic NaCl, which he equated to a loss of weight of the intact gallbladder.

In the rabbit gallbladder, water transport is again linked to active NaCl transport[159], and Leyssac et al.[160] were one of the first investigators to demonstrate that exogenous prostaglandins inhibit fluid absorption and that prostaglandins of the E series were about 100 times more potent than $PGF_{2\alpha}$. In the cat gallbladder, Thornell and Svanvik showed that PGE_2 inhibited fluid absorption, contracted the gallbladder and acted as a choleretic[161]; furthermore, when human gallstones were placed in the cat gallbladder, continuous fluid secretion occurred that was inhibited by indomethacin[162]. Interestingly, VIP-secreting tumours in man produce small bowel

secretion and diarrhea, and VIP similarly induces fluid secretion in the cat and guinea-pig gallbladder[163,164]. As in cat gallbladder, several studies have shown that prostaglandins inhibit fluid absorption and induce secretion in guinea-pig gallbladder[165-167]. Wood et al.[167] found PGE_2 and U-44069, an endoperoxide analogue, to be potent prostaglandins in effecting fluid secretion in the guinea-pig, whereas $PGF_{2\alpha}$, prostacyclin and PGD_2 were considerably less potent.

In the human gallbladder, Rose [168] found a serosa-positive potential difference again due to NaCl absorption. Using the Lucite Ussing chamber, Nahrwold et al.[169] found the absorptive function to be directly related to the histologic abnormality, but this has been questioned. In a recent study by Dumont et al.[170] there was no correlation between histology and absorptive function; more importantly, he found in those gallbladders which initially exhibited secretion into the lumen (4 out of these 11 were hydrops), that indomethacin could reverse this secretion to an absorption. This speaks strongly for prostaglandins inhibiting fluid absorption and inducing intraluminal secretion in animal and human gallbladder.

Gallbladder Motility

Much work has been carried out with CCK and related gastrointestinal peptides and biliary motility; however, their role in contributing significantly to the pathogenesis of cholecystitis and cholelithiasis is debatable. Prostaglandins may play a part in the etiology of cholecystitis and cholelithiasis, but the literature relating prostaglandins to biliary motility is small.

Initial work by Andersson et al.[171] established that

prostaglandins induced guinea-pig gallbladder contractions which were independent of those induced by CCK. It is also established that prostaglandins enhance the contractile response of guinea-pig gallbladder to exogenous acetylcholine or field stimulation[172,173]. Nakata et al.[174] further showed that the guinea-pig gallbladder synthesizes about 4 times as much PGE_2 as $PGF_{2\alpha}$, and suggests that PGE_2 is the main modulator of resting tone and cholinergic-induced contractile responses. PGE and PGF also induce contractions in dog gallbladder[175] and dog cystic duct[176], possibly indicating a role in regulation of bile flow into and out of the gallbladder.

There has been only one report in the literature of the response of human gallbladder to exogenous prostaglandins[167]. This in vitro study met with little success in obtaining consistent responses, which the investigators attributed to the inflammatory changes in the tissue and that prohibited any responses to those added exogenously. It is clear that more work is needed to define the relationship between prostaglandins and human biliary motility.

Prostaglandin Release from the Gallbladder

Much of the evidence presented has suggested a potential role of prostaglandins in the pathogenesis of cholelithiasis, and has stimulated workers to examine prostaglandin release from the gallbladder. Booker and LaMont [177] recently determined release of PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 from guinea-pig gallbladder and did not see the predominance of PGE_2 reported earlier by Nakata[174]. The only investigators to examine prostaglandin release from human gallbladder were Wood and Stamford [178] who found that mucosal extracts contained

PGE, PGF and a compound indistinguishable from PGD_2. They also found that mucosal prostaglandin levels were much higher than that in the muscle and were related to the number of gallstones, postulating that multiple stones traumatize the mucosa and thereby stimulate increased synthesis of prostaglandins.

Indomethacin and Biliary Pain

Several observations about the relationship between prostaglandins and the gallbladder have spurred investigators to look for newer and more effective ways to treat biliary pain. Prostaglandins are pro-inflammatory, produce gallbladder contraction and induce fluid secretion, all of which may aggravate the pain of cholecystitis. This has prompted a Swedish group (Thornell *et al.*[179]) to investigate the effectiveness of intravenous indomethacin in relieving biliary pain in man. In 35 of 37 patients, effective relief was obtained with the average reduction in pain decreasing to two tenths of the original intensity. This single study indicates that prostaglandins play an integral part in mediating the pain of cholecystitis.

OBJECTIVES OF THE STUDY

From the foregoing introduction it is apparent that prostaglandins may play a part in cholelithiasis and cholecystitis. In animal gallbladders, prostaglandins induce intraluminal fluid secretion and muscle contraction; however, the relationship between human gallbladder motility, fluid secretion and prostaglandins is unclear. Additionally, prostaglandins are released from the human gallbladder, but their quantification, and moreover, their exact function in the diseased human gallbladder is not known.

The objectives of this study were:

1. To define the role of prostaglandins in human biliary motility. Since prostaglandins induce guinea-pig gallbladder contraction, it would be helpful to compare prostaglandin-induced changes in human biliary motility results with those of the guinea-pig. These studies would be *in vitro*, as human *in vivo* studies in this aspect of biliary motility would be impractical.
2. To assess and quantify prostaglandin release from the human gallbladder and to relate such data with the contractility studies.

MATERIALS AND METHODS

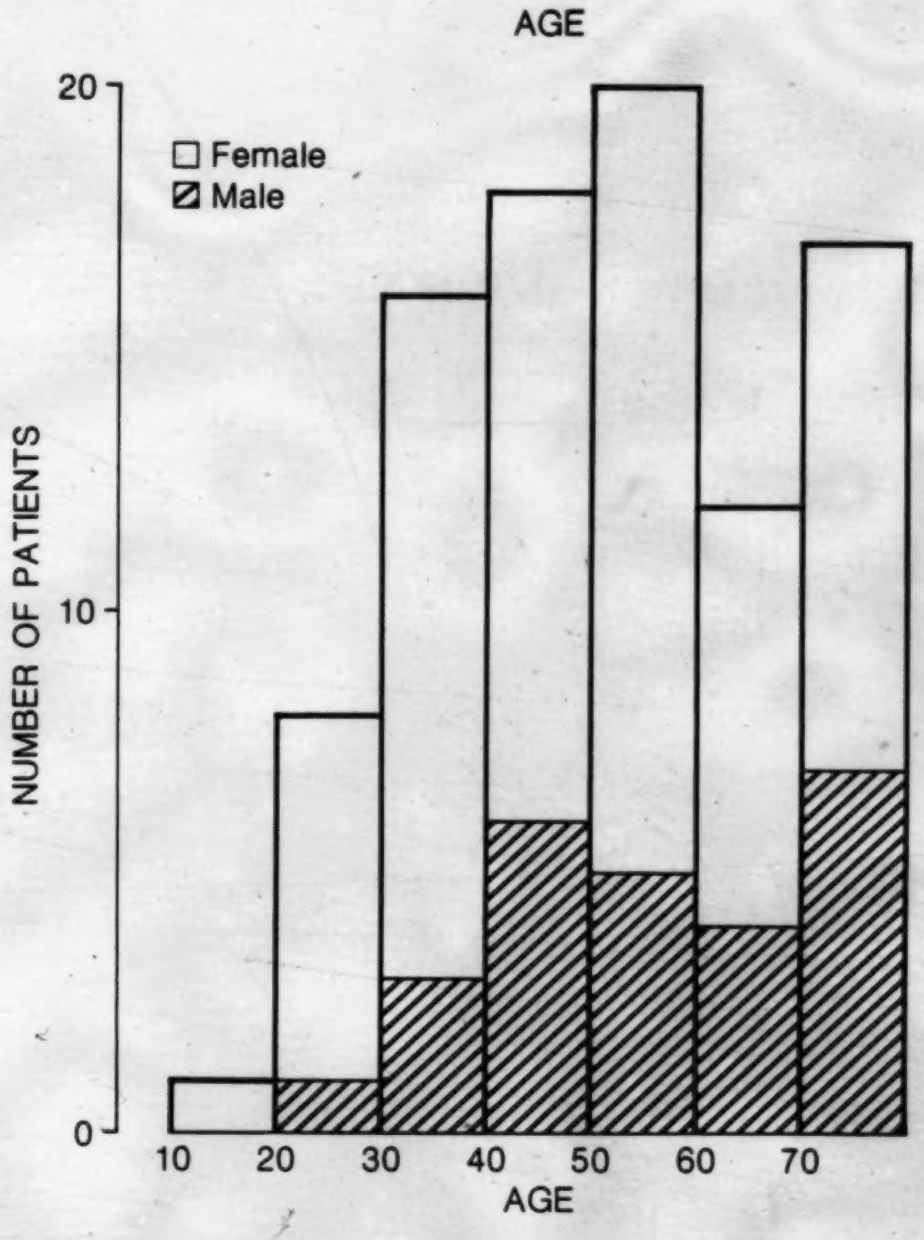
A total of 94 human gallbladders were studied. All cholecystectomies were performed in the University of Alberta, Charles Camsell, Misericordia and Royal Alexandra Hospitals in Edmonton. The cholecystectomies were performed by a variety of general surgeons and there was no preselection of cases. There were 68 female and 26 male patients with a mean age of 51 yr (50 yr for females and 56 yr for males); the ratio of female to male was 2.6 : 1 (Fig. 2).

Stones were multiple in 63 gallbladders and single in 23, giving a ratio of 2.74 : 1. There were 8 acalculous gallbladders, 3 of which had cholesterol polyps. In total, 15 gallbladders (16%) demonstrated cholesterolosis. There were 3 histologically normal gallbladders with no cholesterolosis, although this was not proven by bile analysis. Six gallbladders with hydrops were also studied.

Collection of Human and Guinea-Pig Gallbladders

The specimen was collected immediately upon surgical removal with an ischemia time of less than 20 min. The organ was then placed in oxygenated Krebs solution, examined by the hospital pathologist and opened along its longitudinal axis. A rectangular full-thickness tissue segment, approximately 4 x 2 cm was excised from the anterior wall of the body of the gallbladder and transported in oxygenated Krebs solution to the laboratory. In addition, the hospital chart was consulted for each patient and details regarding the history, clinical presentation, physical findings and laboratory data were noted.

FIGURE 2. Histogram depicting the group of patients from which gallbladders were obtained, with regard to age and sex.


AGE
20
10
0
Female
Male
NUMBER OF PATIENTS
10
20
30
40
50
60
70
AGE

A total of 32 guinea-pig gallbladders were studied. Female guinea-pigs, which weighed between 140 and 220 g, were fasted overnight and killed by cervical dislocation after a single blow to the head. The gallbladder was removed through a midline incision and the entire gallbladder was then promptly placed in Krebs solution.

Preparation of Tissue

A portion of each human gallbladder tissue was processed for histology. The tissue was cut into longitudinal strips, 2 x 10 mm, after excess serosa was trimmed away; care was taken to preserve the entire muscle layer. In similar fashion, guinea-pig gallbladder strips were cut, except that the entire wall was used for study. All contractility studies were started within 1 h of the cholecystectomy.

Histology

Tissues for light microscopy were fixed for 24 h in 10% neutral buffered formalin and embedded in paraffin (Tissue-prep; Fisher Scientific Ltd., Edmonton). Transverse sections, 8 μm thick, were floated onto albumen-coated slides and stained with hematoxylin and eosin.

The slides were then examined without knowledge of the motility study results. A grading was assigned to several histological parameters of the transverse gallbladder wall - inflammatory infiltrate, fibrosis, hyperemia and edema in each of the three layers of the wall - mucosa, muscularis and areolar layer (Fig. 3). These were scored 0, normal; 1, definite but mild histopathology; and 2, advanced histopathology. An additional score of 2 was assigned if the mucosa was

HISTOLOGICAL SCORING SHEET

Score

Mucosa (incl. lamina propria)

- ulceration
- thickness
- infiltrate: lymphocytic
  neutrophilic
  eosinophilic
  plasma cells
- RBC's
- edema

Muscle Layer

- thickness
- infiltrate: lymphocytic
  neutrophilic
  eosinophilic
  plasma cells
- RBC's
- fibrosis
- edema

Areolar Layer

- infiltrate: lymphocytic
  neutrophilic
  eosinophilic
  plasma cells
- RBC's
- fibrosis
- edema

Total Score _____

FIGURE 3

absent (ulceration) because of advanced disease.

All human gallbladders were classified as chronic mild (CMC), chronic advanced (CAC) or acute (AC) cholecystitis. This was performed by both an overall impression and objectively, according to the histologic score. A total score of 0 to 5 was placed in the CMC category; 6 to 12 in the CAC and 13 and above in the AC categories. Two other categories were added depending upon clinical presentation and gross features - normal and hydrops. Guinea-pig gallbladder was assumed to be normal and was therefore not submitted for histological examination.

Drugs

The Krebs solution that continuously bathed the tissue was at physiologic pH (7.4) and was made up in double distilled de-ionized water. It had the following constituents (mM): NaCl 116; KCl 5.4; $CaCl_2$ 1.2; NaH_2PO_4 1.2; $NaHCO_3$ 22; $MgCl_2.6H_2O$ 1.2; and D-glucose 10.1.

Drugs used and their sources were as follows: PGE_1, PGE_2, $PGF_{2\alpha}$, PGD_2, PGB_2, arachidonic acid, acetylcholine chloride, indomethacin, cholecystokinin-octapeptide (amide sulfated) (Sigma Chemical Co., St. Louis, Mo.); endoperoxide analogue U-44069 (The Upjohn Co., Kalamazoo, Mich.); prostacyclin (Dr. J. Scholtholt, Hoechst AG, Frankfurt, Germany); leukotriene (LT) B_4, LTC_4, LTD_4 (Dr. J. Rokach, Merck Frosst Canada Inc., Pointe Claire-Dorval, Quebec); calcium ionophore A23187 (Calbiochem-Behring, San Diego, CA); LTC_4 and LTD_4 antagonist FPL55712 (Fisons Pharmaceutical Laboratories). Stock solutions (10^{-1} or 10^{-2} M) of the prostaglandins were dissolved in 99% ethanol and stored at -20°C. Prostacyclin was dissolved in distilled de-ionized water,

maintained at pH 10 with $NaHCO_3$ and stored at -20°C. Leukotrienes were dissolved in 99% ethanol and stored at -70°C. Acetylcholine was stored at -20°C in the anhydrous state. All drugs were diluted with distilled water to the desired concentration range before each experiment. Due to the marked instability of prostacyclin in solution (half-life = 3 min), each individual concentration was prepared in water immediately prior to its addition to the organ bath.

Motility Studies

Gallbladder strips were mounted along their longitudinal axis in 5 ml organ baths. One end of each strip was attached with a 4-0 silk ligature to a fixed hook at the bottom of the bath, and the other end was similarly attached to an isometric tension transducer. The bath contained Krebs solution maintained at 37°C and was continuously bubbled with 95% O_2 and 5% CO_2. Half of the strips were incubated in Krebs containing indomethacin (10^{-5} M).

Isometric contractions in response to the application of drugs were measured with force-displacement transducers (FT.03C; Grass Instrument Co., Quincy, Mass.) connected to a Beckman polygraph recorder. The change in tension in g from the baseline was taken as a response to the concentration of the drug in the organ bath at the time the response occurred.

The strips were adjusted to an initial tension of 0.5 g in the case of human tissues and 1.0 g in the case of guinea-pig tissues. During the 40 min equilibration period, they were washed continuously with Krebs solution at 5 ml/min. Most strips maintained a stable baseline tension; in those demonstrating a spontaneous rise in tension, it was manually

readjusted to 0.5 g and 1.0 g in human and guinea-pig muscle strips, respectively. Each strip was exposed to a maximally effective concentration of acetylcholine (Ach 10^{-3} M), both after the initial 40 min and at the end of the experiment. The Ach concentration at the beginning served as a test of viability and also appeared to stimulate the tissue; that at the end provided a contraction for comparative purposes. After this initial stimulation, 30 min were allowed to elapse before prostaglandins were added.

Application of Drugs

Each agonist was tested on at least two and as many as eight strips; half of the strips were in the absence and half in the presence of indomethacin (10^{-5} M). Strips were exposed to increasing concentrations of each drug until the maximal response was obtained. In this fashion a non-cumulative concentration-response curve was constructed. The drug was washed out of the bath by overflow with fresh, warmed and oxygenated Krebs solution before addition of the next concentration. Similar waiting periods were allowed between additions of increasing concentrations of each drug.

Analysis of Data

For each drug tested, contractile responses were expressed as a percent of the maximal contraction of each strip. In some instances, the contractile responses were expressed as a percent of the response to 10^{-3} M Ach. These values were plotted as concentration-response curves and the ED_{50} value (agonist concentration producing half the maximal response) was calculated for each concentration-response curve of each

gallbladder strip; the geometric mean ED_{50} was then calculated and expressed in appropriate units with the 95% confidence limits. The ED_{50} value could only be obtained when a maximum response was achieved with a particular agonist.

Prostaglandin Release

Prostaglandin release from human gallbladder was examined in both spontaneous and stimulated states. Stimulus-induced prostaglandin release was tested in the presence of three agents - calcium ionophore A23187, Ach and cholecystokinin-octapeptide (CCK-OP). Calcium ionophore causes an increase in intracellular calcium that interacts with phospholipase A_2; this releases membrane-bound arachidonate and thereby stimulates prostaglandin biosynthesis. Ach and CCK-OP were tested as they may be more "physiologic" stimulants of prostaglandin release.

Separation of prostaglandins and leukotrienes and their detection were accomplished by reverse-phase high performance liquid chromatography (HPLC). Fresh human gallbladder tissue was obtained as previously documented and the serosa was trimmed and discarded, leaving the mucosa and muscularis intact. The tissue was cut into 2 mm square fragments and suspended in flasks containing 20 ml of oxygenated Krebs' solution. The flasks were inserted into a shaking water bath at 37°C for 15 min. The tissue was stimulated for 15 min with calcium ionophore at a concentration of 5.7 μM (3 μg/ml). The suspension was filtered and 80 ml of 95% ethanol was added. The fluid was centrifuged at 13,000 rpm and the ethanol layer was collected and evaporated at 55°C with a Brinkman R110 Rotavapor. The residual fluid was adjusted to pH 3 and purified through a Waters C_{18} SEP-PAK column that was washed with 40 ml

water and washed a further three times with 10 ml methanol; the methanol was then evaporated under a stream of N_2 to produce a residue.

For prostaglandin assays, 1 ml of acetonitrile, 2 µl of diisopropylethylamine and 3 M excess dibromoacetophenone were added to the residues and passed through a Bondapak C_{18} column at a rate of 1.2 ml/min with 55% acetonitrile and 45% water; this was controlled by a 650 Solvent Programmer. Prostaglandins were detected by a Waters Fixed Wavelength 441 Absorbance Detector at 254 nm and integrated by a 3390A Hewlett Packard Integrator.

For leukotriene assay, the residues were dissolved in 500 µl of methanol. The same equipment and procedure were used but the solvents were 65% methanol, 35% water and 0.001% acetic acid adjusted to a pH of 5.5 with NH_4OH. Detection of leukotrienes was at 280 nm.

RESULTS

Histology

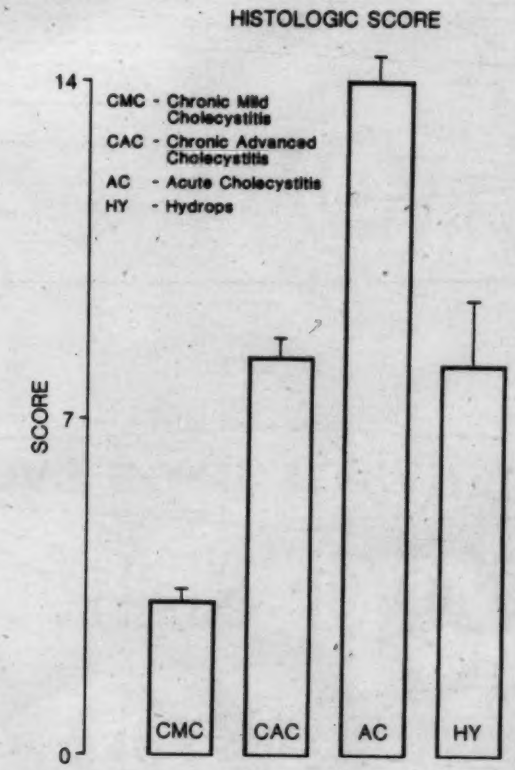
All gallbladders were classified as chronic mild, chronic advanced or acute cholecystitis according to the histologic score (Fig. 3). Fig. 4 illustrates that those gallbladders with AC had the highest histologic score; also, those with hydrops had a score not significantly different from those with CAC. The disease distribution is shown in Fig. 5 with the CMC being the commonest and the AC and hydrops gallbladders being the least common disease entities.

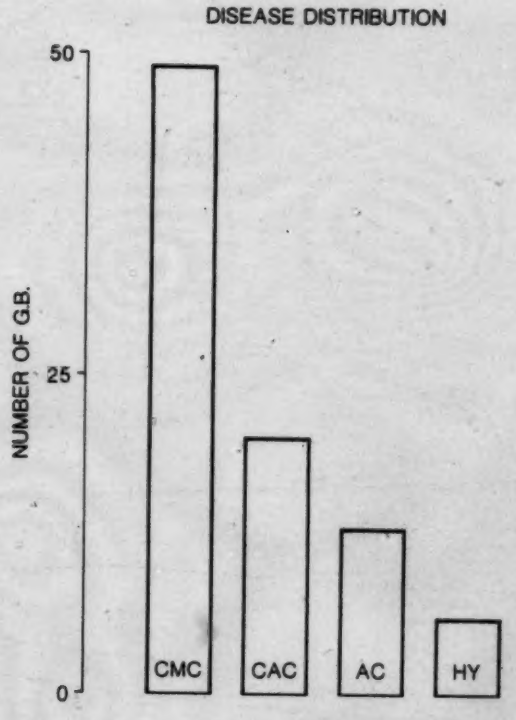
Histologically, gallbladders with CMC (n = 50) demonstrated mucosal hyperplasia and early fibrotic changes in all three layers. The muscle layer was well preserved; in the mucosa and serosa the inflammatory infiltrate was scant - mostly lymphocytic with sparse plasma cells. Gallbladders with CAC (n = 20) were much thicker. The predominant finding was fibrosis in all three layers; in the majority, the muscle layer was the widest with collagen bundles infiltrating between the muscle fibres, and in a few the muscle was almost totally replaced by fibrosis. There was thus a large variability in the relative amounts of muscle and collagen fibres. The mucosa revealed atrophy and ulceration and the cell infiltrate was more visible; this comprised mostly lymphocytes, plasma cells and occasional eosinophils. This chronic cell infiltrate was also scattered throughout the wall. Rokitansky-Aschoff sinuses were evident in the mucosa. Mild hyperemia was noted in the serosa.

Those gallbladders with AC (n = 15) had extensive disease in all layers. On gross examination, the serosa was hemorrhagic and edematous

FIGURE 4. Histogram showing the mean histologic score (± SEM) in human gallbladders with chronic mild cholecystitis (CMC), chronic advanced cholecystitis (CAC), acute cholecystitis (AC) and hydrops (HY).

FIGURE 5. Histogram showing the disease distribution in all the pathologic human gallbladders obtained.


HISTOLOGIC SCORE
CMC - Chronic Mild Cholecystitis
CAC - Chronic Advanced Cholecystitis
AC - Acute Cholecystitis
HY - Hydrops
SCORE
14
7
0
CMC
CAC
AC
HY


DISEASE DISTRIBUTION
NUMBER OF G.B.
50
25
0
CMC
CAC
AC
HY

and the entire organ was thickened. Microscopically, hyperemia, red blood cell extravasation and edema were seen in all layers, but more so in the mucosa and serosa. With severe disease, the hemorrhagic findings were marked. Ulceration of the mucosa was prominent and an acute cell infiltrate of lymphocytes and polymorphonuclear leukocytes was seen throughout the wall. The leukocytes were mostly seen around blood vessels indicating previous margination. Five out of the 15 gallbladders with AC revealed muscle hypertrophy. As in the chronic advanced disease, fibrosis was present in all layers, sometimes being extensive. The 6 gallbladders with hydrops showed virtually no mucosal cells due to the pressure effect and no inflammatory cell infiltrate. Surprisingly, muscle hypertrophy was present in 3 of the 6 gallbladders but fibrosis was the predominant feature in all.

Clinical Data

In general, the history, physical findings and laboratory data were not highly specific for those patients with acute cholecystitis. The mean age of these 15 patients was 58 yr, somewhat higher than the entire group and the female to male ratio was similar. Seven of the 15 had significant pain and only 3 had prominent physical findings. Of the five laboratory parameters examined - leukocyte count, serum oxalacetic transaminase, lactic dehydrogenase, alkaline phosphatase and bilirubin - only 4 patients had an elevation of any two of these parameters. Only one case had cystic duct obstruction intraoperatively. Interestingly, 6 patients had gallstone pancreatitis with a markedly elevated serum amylase.

MOTILITY EFFECTS

Spontaneous Activity

After the tissues were mounted in organ baths, the strips demonstrated intrinsic contractile and relaxant activity - spontaneous activity; representative recordings are shown in Fig. 6. Spontaneous activity occurred in less than half of the human gallbladder strips. The frequency of spontaneous activity was no more than 2 to 3 per min, and the amplitude varied considerably; from 10 to as much as 80% of the maximal contraction to 10^{-3} M Ach. The initial concentration of Ach (10^{-3} M) to the muscle strip in many cases initiated spontaneous activity which persisted throughout the experiment.

Only when spontaneous activity was marked (Fig. 6, C and D), did it mask the prostaglandin-induced response of a muscle strip. In general, a prostaglandin-induced contractile response reduced or abolished the spontaneous activity. Occasionally, with each of the prostaglandins, there was a mild enhancement of spontaneous activity near maximal contraction of a muscle strip. The percentage of strips displaying spontaneous activity in the three disease groups were CMC - 43.4%, CAC - 26% and AC - 36.7% (Fig. 7A). In gallbladders with AC, the percentage of strips demonstrating marked spontaneous activity did not comprise a larger percent than those strips from gallbladders with CMC and CAC. In comparison, 28.6% of guinea-pig muscle strips demonstrated spontaneous activity, although the amplitude was smaller than that of human gallbladder strips, varying from 5 to 30% of the maximal contraction to 10^{-3} M Ach.

Indomethacin (10^{-5} M) when added to the organ bath suppressed or

FIGURE 6. Representative recordings of spontaneous activity in tissues from gallbladders with chronic mild, chronic advanced and acute cholecystitis. A and B. Muscle strips showing minimal to moderate spontaneous activity. C and D. Muscle strips with marked spontaneous activity. Recordings are obtained randomly from different gallbladder strips..

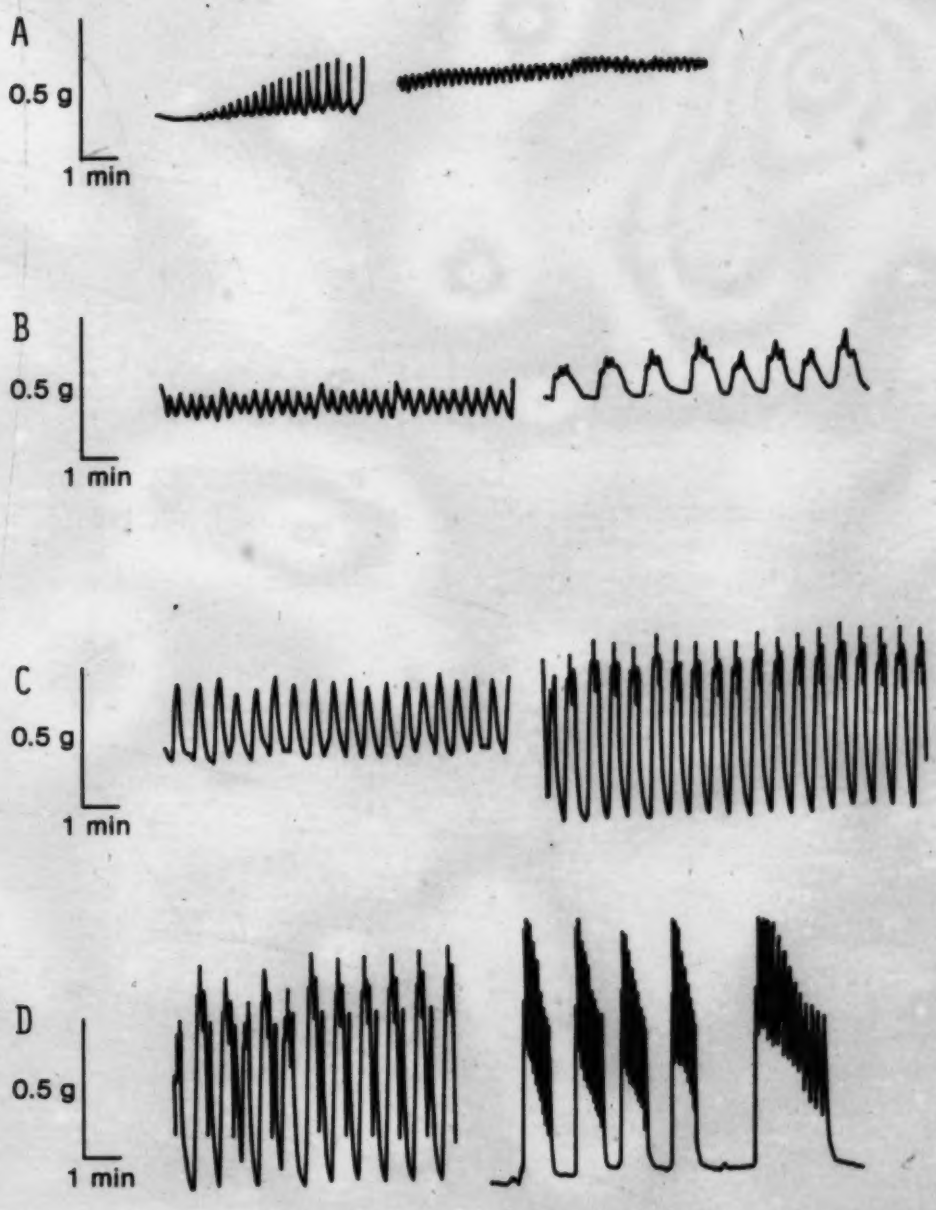
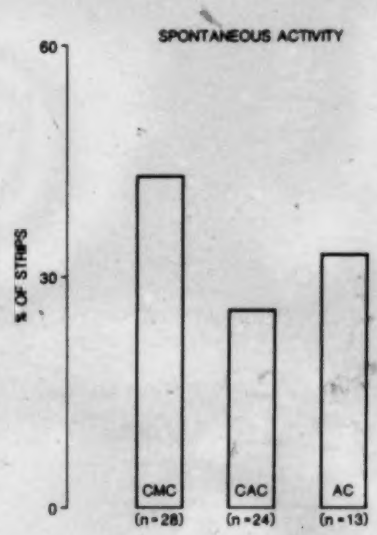
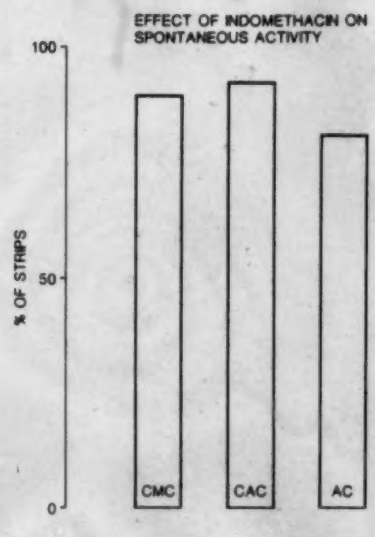

A
0.5 g
1 min
B
0.5 g
1 min
C
0.5 g
1 min
D
0.5 g
1 min

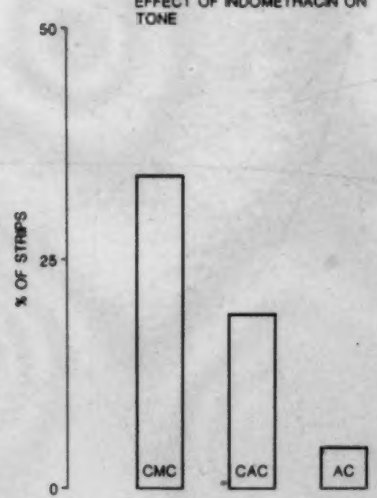
FIGURE 7A. Percentage of human muscle strips displaying spontaneous activity in gallbladders with chronic mild, chronic advanced and acute cholecystitis.

FIGURE 7B. Percentage of human muscle strips with spontaneous activity showing an inhibition of spontaneous activity by 10^{-5} M indomethacin.

FIGURE 7C. Percentage of human muscle strips showing an effect of 10^{-5} M indomethacin on muscle tension.


SPONTANEOUS ACTIVITY
% OF STRIPS
60
30
0
CMC
CAC
AC
(n=28)
(n=24)
(n=13)


EFFECT OF INDOMETHACIN ON SPONTANEOUS ACTIVITY
% OF STRIPS
100
50
0
CMC
CAC
AC


EFFECT OF INDOMETHACIN ON TONE
% OF STRIPS
50
25
0
CMC
CAC
AC

completely abolished spontaneous activity in those human muscle strips displaying spontaneous activity. The percentage of strips demonstrating an inhibition of spontaneous activity by 10^{-5} M indomethacin in each disease group were CMC - 38.9%, CAC - 24.1% and AC - 29.7% (Fig. 7B).

Muscle Tension

The initial tension on human muscle strips was set at 0.5 g. During the initial 40 min equilibration period the tension increased spontaneously in the majority of strips. In general, those strips with larger Ach responses and marked spontaneous activity demonstrated a larger increase in tension. To assess the effect of indomethacin on muscle tension, a positive effect was defined as a decrease in tension of 0.05 g (equivalent to one-tenth of the initial baseline tension). In each disease group, the percentage of strips demonstrating an effect of indomethacin on muscle tension were CMC - 33.8%, CAC - 18.8% and AC - 4.8% (Fig. 7C).

Endogenous Tone

Prostaglandin-induced endogenous tone is a reflection of the prostaglandin-induced responses due to endogenous levels of prostaglandins present in the tissue strips. In the CMC and CAC groups, PGE_1 and PGE_2 in 141 strips (n = 34 gallbladders) caused concentration-dependent contractions in 112 (79.4%). In the remaining 20.6%, spontaneous activity was marked (Fig. 6, C and D) and inconsistent or no responses were produced. With indomethacin (10^{-5} M) added to the bath thereby suppressing spontaneous activity, concentration-dependent contractions to PGE_1 and PGE_2 were seen in all 141 strips. However,

$PGF_{2}\alpha$, PGD_2, PGB_2, and U-44069 produced concentration-dependent contractions in 100% of strips, regardless of marked spontaneous activity, in the presence and absence of indomethacin.

In those gallbladders with AC, PGE_1 and PGE_2 produced either no response or small relaxations, both in the presence and absence of indomethacin 10^{-5} M (n = 3 gallbladders). In the same gallbladders, $PGF_{2}\alpha$ produced concentration-dependent contractions in 1 out of 6 strips without indomethacin, and 6 out of 6 strips with indomethacin.

DRUG INDUCED CHANGES IN MOTILITY

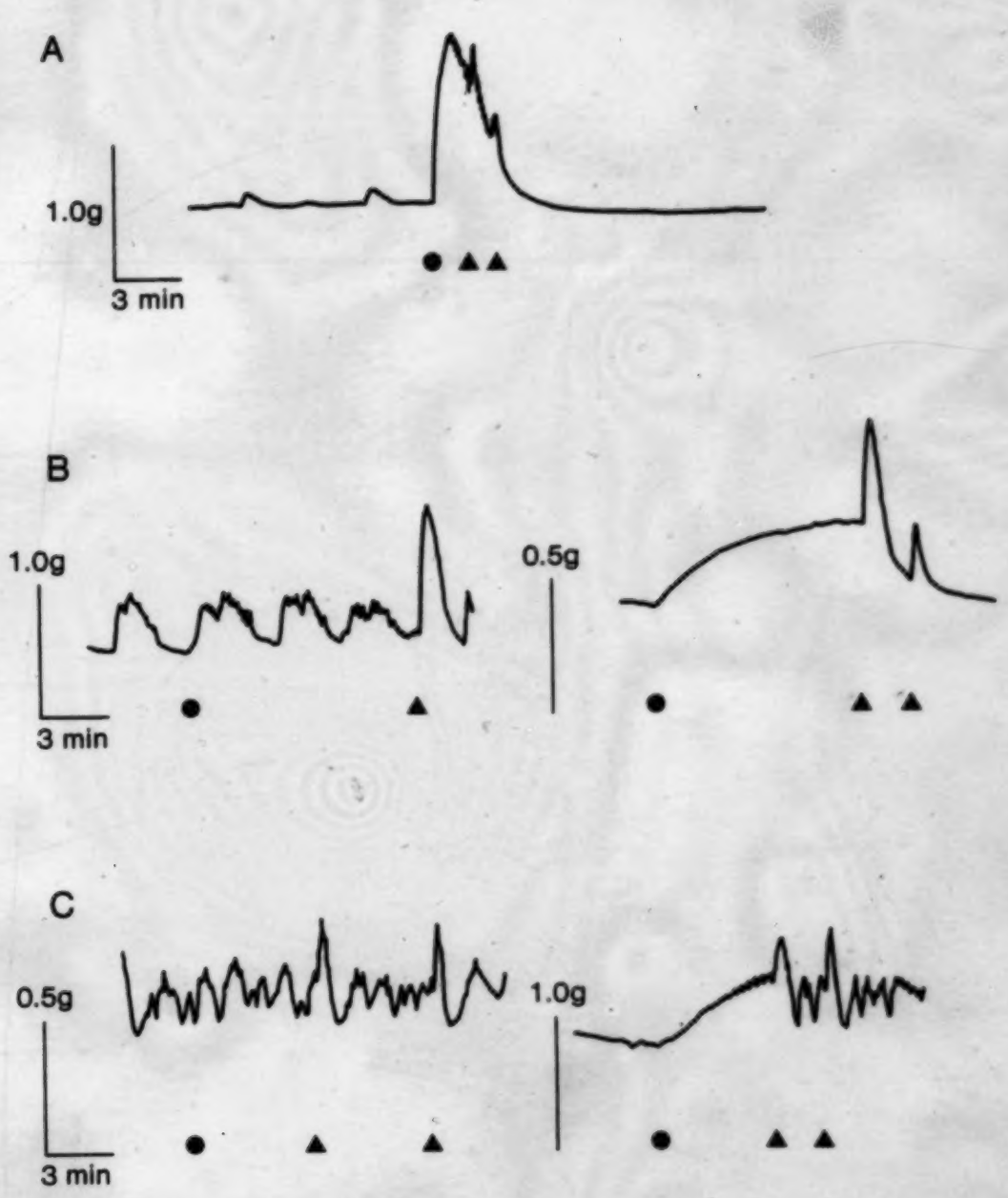
Chronic Mild and Chronic Advanced Cholecystitis

Prostaglandins and leukotrienes were added exogenously to human, and in some cases guinea-pig muscle strips. Since prostaglandin responses in gallbladders with AC were inconsistent even in the presence of indomethacin (see below), the majority of all motility studies were in those gallbladders with CMC and CAC. In these latter categories, concentration-dependent contractions were seen with most prostaglandins when the strips were incubated with indomethacin; in the absence of indomethacin inconsistent contractile responses, small relaxations or no responses were frequently seen, especially in the presence of marked spontaneous activity (Fig. 8, B and C). When concentration-dependent contractions were seen in the absence of indomethacin, it was present in almost twice as many strips from gallbladders with CMC than from gallbladders with CAC. The magnitude of contractile response of the tissues to the various prostaglandins when incubated without

FIGURE 8A. Contractile responses in human gallbladder to 10^{-3} M acetylcholine. Circles denote addition of a particular agonist to the organ bath and triangles denote subsequent washings.

FIGURE 8B. The tracing on the left shows no contractile response to 10^{-8} M PGE_1 in the presence of marked spontaneous activity. That on the right illustrates a contractile response to the same concentration of PGE_1 in the presence of no spontaneous activity.

FIGURE 8C. The tracing on the left shows no contractile response to 10^{-7} M PGE_2 in the presence of marked spontaneous activity. That on the right illustrates a contractile response to the same concentration of PGE_2 in the presence of minimal spontaneous activity. All tracings are from muscle strips of different gallbladders with chronic mild or advanced cholecystitis.


A
1.0g
3 min
B
1.0g
3 min
0.5g
C
0.5g
3 min
1.0g

indomethacin was less than when the tissues were incubated with indomethacin; however, the sensitivity of the tissue to the various prostaglandins was not changed by the presence of indomethacin. Therefore, concentration-response curves to prostaglandins were measured in those strips incubated with indomethacin only. Examples of contractile responses to the various prostaglandins and leukotrienes in human and guinea-pig gallbladder are illustrated in Figures 9 and 10, respectively. It is evident that the rate of contraction to prostaglandins in human tissue is much less than that in guinea-pig tissue. Also, the contractile responses to the various prostaglandins in human tissue are quite similar, but in guinea-pig, the contractile responses to leukotrienes are quite different than those to PGD_2 and prostacyclin.

Motility studies in three histologically normal gallbladders revealed that the contractile responses to PGE_2 in the absence of indomethacin were of the same magnitude as those in the presence of indomethacin. The contractile responses to PGE_1, however, were augmented when the tissues were incubated with indomethacin. Responses to $PGF_2\alpha$, U-44069 and PGI_2 were similar to those in gallbladders with CMC and CAC (see below).

The presence of gallstone pancreatitis did not affect the motility results in the various disease groups.

Comparison of Acetylcholine and PGE Responses

The mean Ach response for each strip at the end of the experiment was calculated in each disease group and the results are expressed in Table 1. As can be seen from the 95% confidence limits, the Ach

FIGURE 9. Representative tracings of contractile responses in human gallbladder strips to $PGF_{2\alpha}$ (10^{-6} M, panel A), PGD_2 (10^{-5} M, panel B), PGB_2 (10^{-6} M, panel C) and U-44069 (10^{-6} M, panel D). Circles denote addition of a particular agonist to the organ bath and triangles denote subsequent washings.

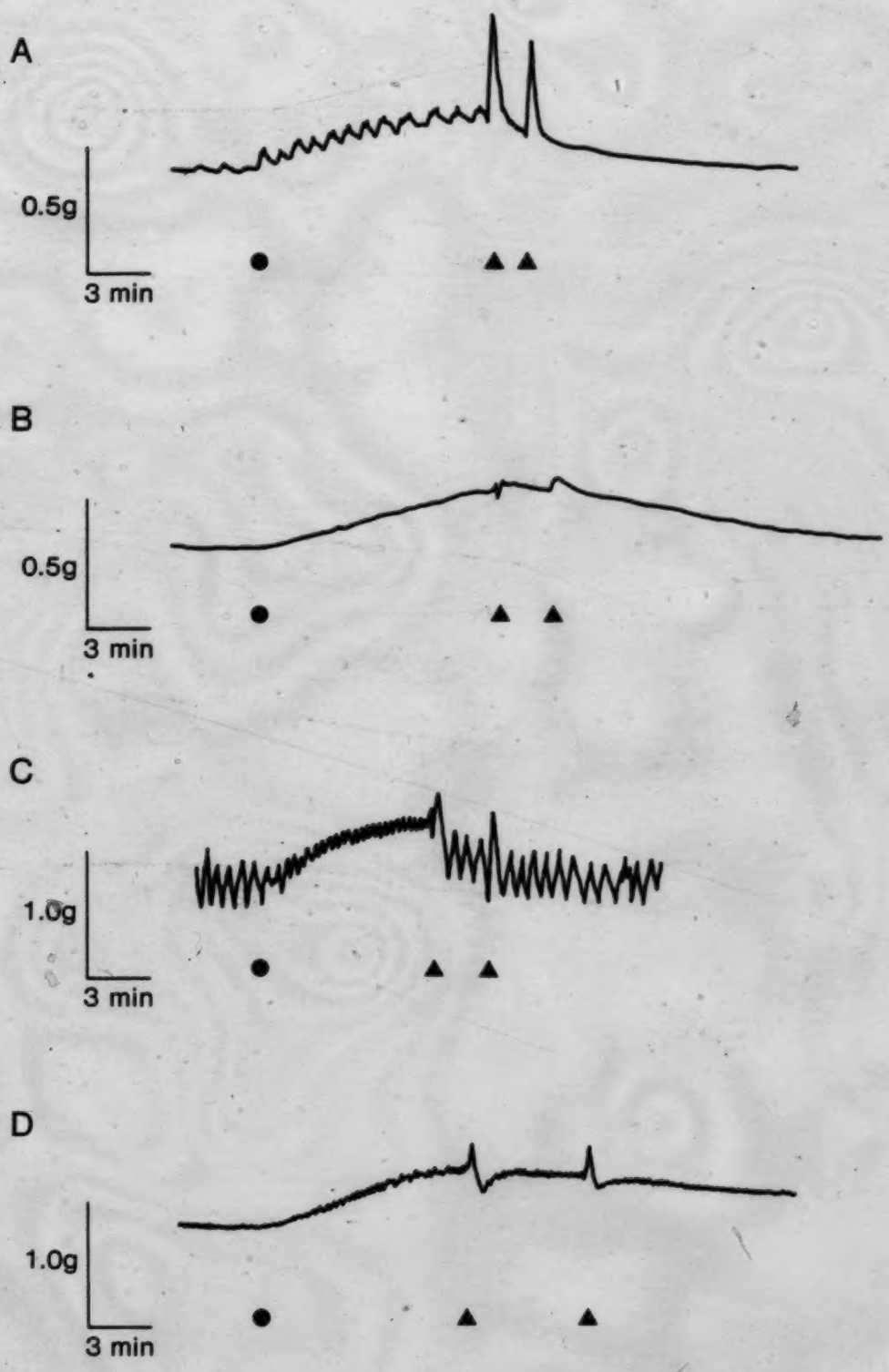

A
0.5g
3 min
B
0.5g
3 min
C
1.0g
3 min
D
1.0g
3 min

FIGURE 1Q. Representative tracings of contractile responses in guinea-pig gallbladder to PGD_2 (3×10^{-5} M, panel A), prostacyclin (2.86×10^{-7} M, panel B), LTC_4 (10^{-7} M, panel C) and LTD_4 (10^{-7} M, panel D). Circles denote addition of a particular agonist to the organ bath and triangles denote subsequent washings. Note the gradual return to baseline in guinea-pig gallbladder responses to leukotrienes despite repeated washings.

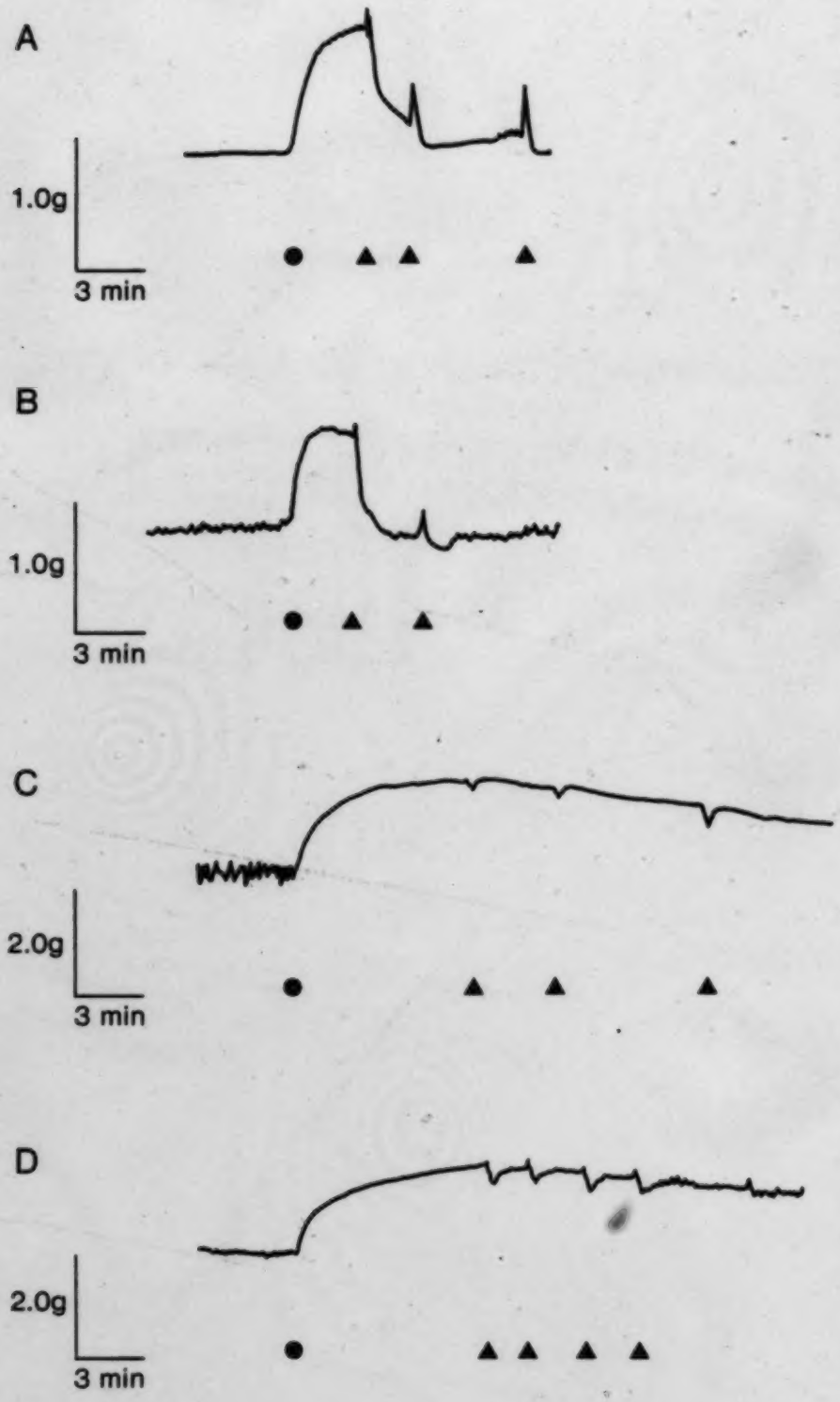

A
1.0g
3 min
B
1.0g
3 min
C
2.0g
3 min
D
2.0g
3 min

TABLE 1

Maximal Acetylcholine Responses (10^{-3}M)
in the Various Disease Groups of Human Gallbladder

Group	Mean (g)	95% Confidence Limits
1. CMC (n=38)	1.66	1.40 - 1.91
2. CAC (n=18)	1.72	1.22 - 2.22
3. AC (n=13)	0.84	0.59 - 1.17
4. HYDROPS (n=6)	0.24	0.08 - 0.41
5. NORMAL*	0.86	0.39 - 1.34

* includes those gallbladders with no cholesterol stones (mixed or pure), but with pigment stones only, cholesterolosis or cholesterol polyps

responses in the CMC and CAC were not significantly different from each other ($P > 0.05$); in contrast, the responses in the AC group were much less and were significantly different ($P < 0.05$) from those of the CMC and CAC gallbladders. Interestingly, the responses in the "normal" group are approximately half that of the CMC group.

The mean maximal responses to PGE_1 and PGE_2 in the CMC and CAC groups were 0.42 and 0.53 g respectively (Fig. 11), and were not significantly different ($P > 0.05$). As seen in Fig. 12, PGE_1 and PGE_2 responses (CMC and CAC) were 22.2 and 30.3% respectively, of the Ach response in paired muscle strips. As Fig. 8 depicts, Ach and PGE contractile responses of human gallbladder muscle strips are quite different. Ach induces a contraction with an immediate onset and a rapid rise to a peak within 1 min; PGE_1 and PGE_2 induce contractions with a much slower rate of rise and have a sustained maximal effect and also a more gradual return to baseline tension. Fig. 13 illustrates the relationship ($r = 0.588$ not significant) between the Ach versus the PGE_1 and PGE_2 responses; as the Ach response increases so also does the prostaglandin response.

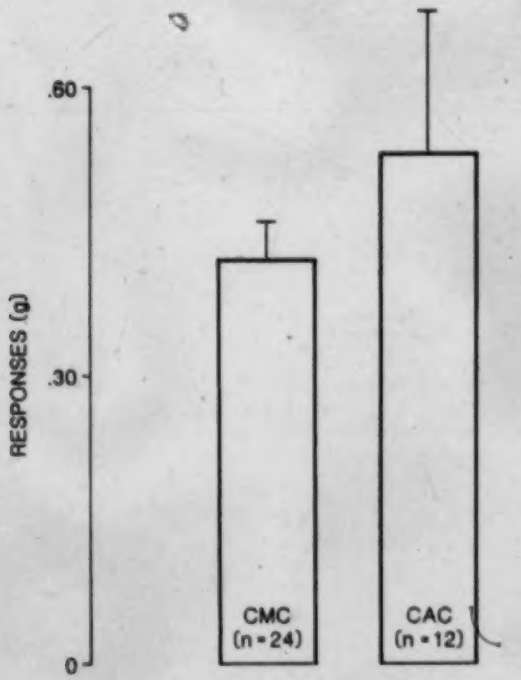
Prostaglandin E_1 and E_2

PGE_1 and PGE_2 produced concentration-dependent contractions in human gallbladders with CMC and CAC in the presence of indomethacin (Fig. 14). The threshold response occurred between 10^{-12} and 10^{-11} M and the maximum response occurred at 10^{-6} M with both PGE_1 and PGE_2. The maximal responses to PGE_1 and PGE_2 were not significantly different. The ED_{50} (95% confidence limits) for PGE_1 and PGE_2 were 25.9 nM (15.9 - 42.3) and 23.3 nM (13.1 - 41.6), respectively.

FIGURE 11. Combined PGE_1 and PGE_2 mean maximal responses (g ± SEM) in human gallbladders with chronic mild and advanced cholecystitis.

FIGURE 12. PGE_1 and PGE_2 mean maximal responses (g ± SEM) in human gallbladders with chronic mild and advanced cholecystitis (combined) and their corresponding acetylcholine (10^{-3} M) responses.

PGE$_1$ AND PGE$_2$ RESPONSES



ACH AND PGE RESPONSES
- CMC & CAC

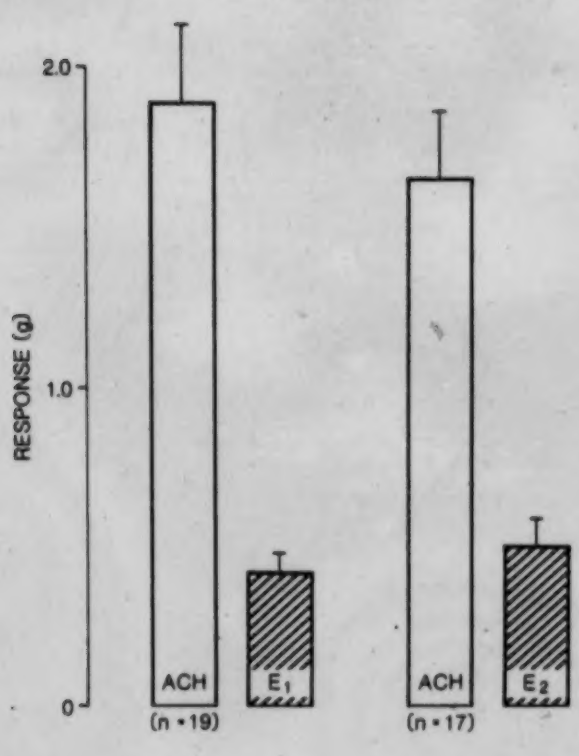


FIGURE 13. Linear regression analysis of acetylcholine (10^{-3} M) versus PGE_1 and PGE_2 mean maximal responses in the presence of 10^{-5} M indomethacin (correlation coefficient = 0.588).

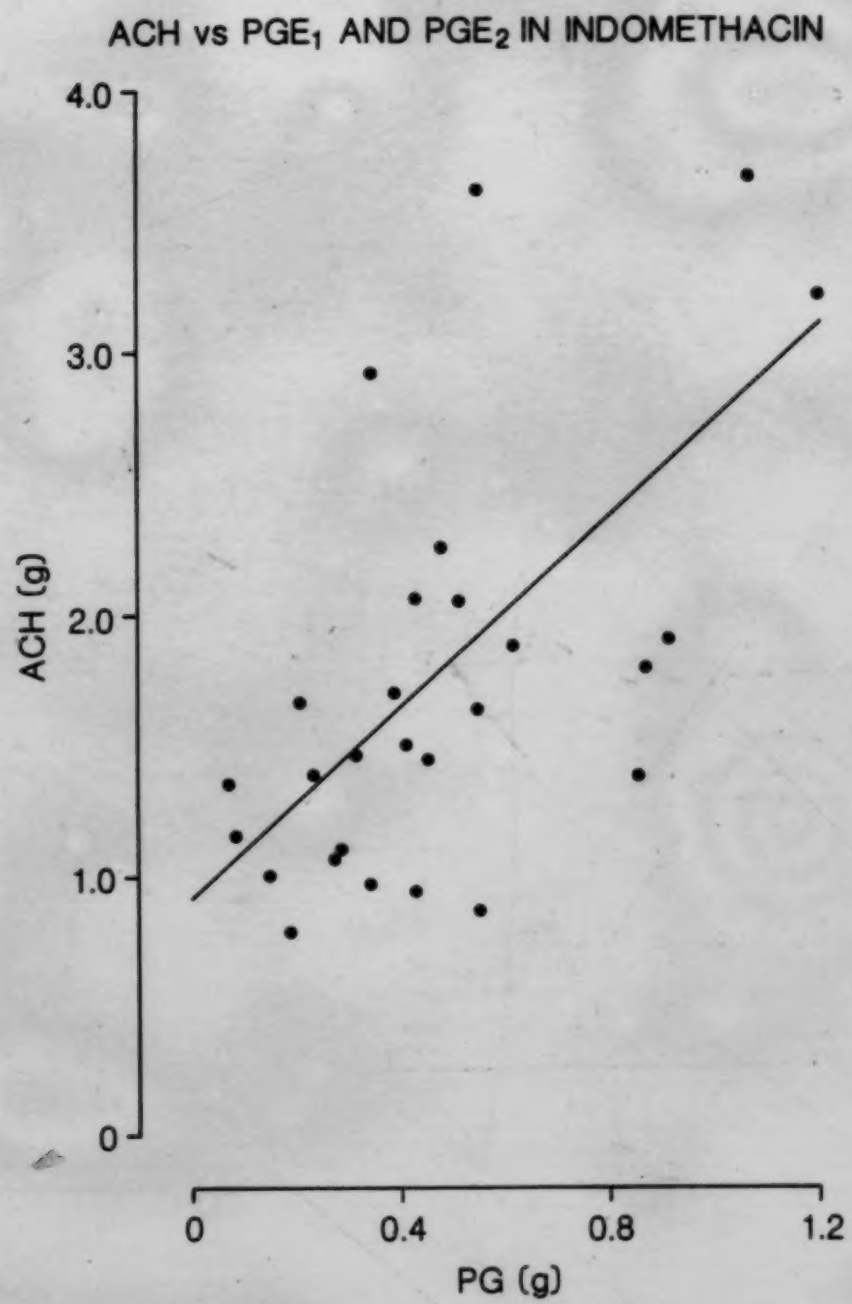
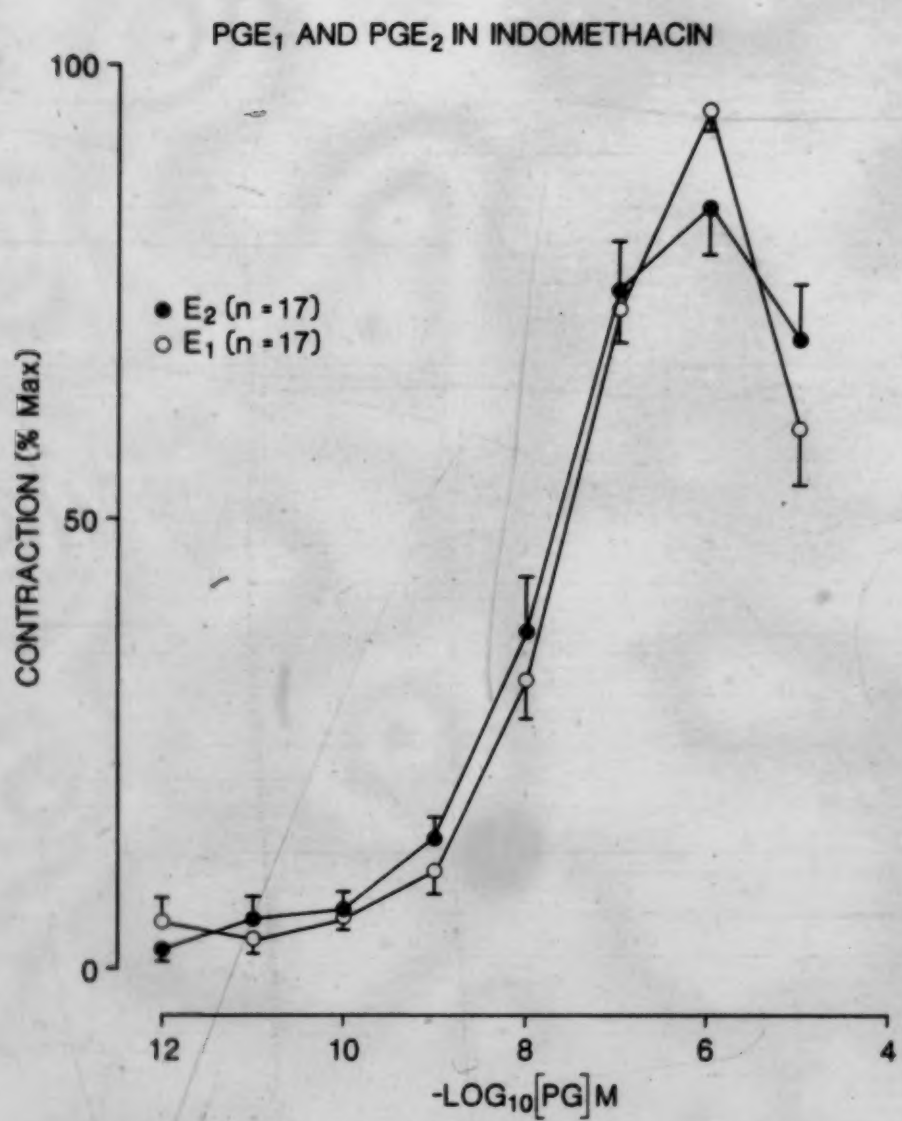

ACH vs PGE1 AND PGE2 IN INDOMETHACIN
4.0
3.0
2.0
1.0
0
ACH (g)
0
0.4
0.8
1.2
PG (g)

FIGURE 14. Concentration-response curves for PGE_1 and PGE_2 in human gallbladders with chronic mild and advanced cholecystitis in the presence of 10^{-5} M indomethacin. Points represent the mean ± SEM for n observations. Results are expressed as percentages of the maximal response for each agonist.


PGE1 AND PGE2 IN INDOMETHACIN
100
50
0
CONTRACTION (% Max)
● E2 (n = 17)
○ E1 (n = 17)
12
10
8
6
4
-LOG10[PG]M

Prostaglandin $F_{2\alpha}$ and Prostaglandin D_2

$PGF_{2\alpha}$ and PGD_2 produced concentration-dependent contractions in human gallbladders with CMC and CAC in the presence of indomethacin (Fig. 15). The threshold response for $PGF_{2\alpha}$ was between 10^{-9} and 10^{-8} M and that for PGD_2 was between 10^{-8} and 10^{-7} M. A maximum response to $PGF_{2\alpha}$ and PGD_2 was not obtained at concentrations as high as 10^{-5} M and therefore ED_{50} values could not be calculated. However, the potencies of $PGF_{2\alpha}$ and PGD_2 when expressed both as a percent of their maximum response (Fig. 15) and as a percent of the maximal response to 10^{-3} M acetylcholine (Fig. 16), are much less than that of PGE_2. It is thus evident that $PGF_{2\alpha}$ and PGD_2 are 100 to 1000 times less potent than PGE_2 in effecting human gallbladder muscle contraction.

U-44069 and Prostaglandin B_2

U-44069 and PGB_2 produced concentration-dependent contractions in human gallbladders with CMC and CAC in the presence of indomethacin (Fig. 17). The threshold response for U-44069 was between 10^{-10} and 10^{-9} M. A maximum response with U-44069 was not obtained but, as Figure 17 illustrates, it is less potent than PGE_2. The threshold response for PGB_2 was between 10^{-10} and 10^{-9} M and the maximum response was at 10^{-4} M. The ED_{50} (95% confidence limits) for PGB_2 was 2350 nM (760-7250), which is over 100 times that of PGE_2 and is significantly different ($P < 0.05$).

Prostaglandin D_2

PGD_2 produced concentration-dependent contractions in both human

FIGURE 15. Concentration-response curves for PGE_2, $PGF_{2\alpha}$ and PGD_2 in human gallbladders with chronic mild and advanced cholecystitis in the presence of 10^{-5} M indomethacin. Points represent the mean ± SEM for n observations. Results are expressed as percentages of the maximal response for each agonist.

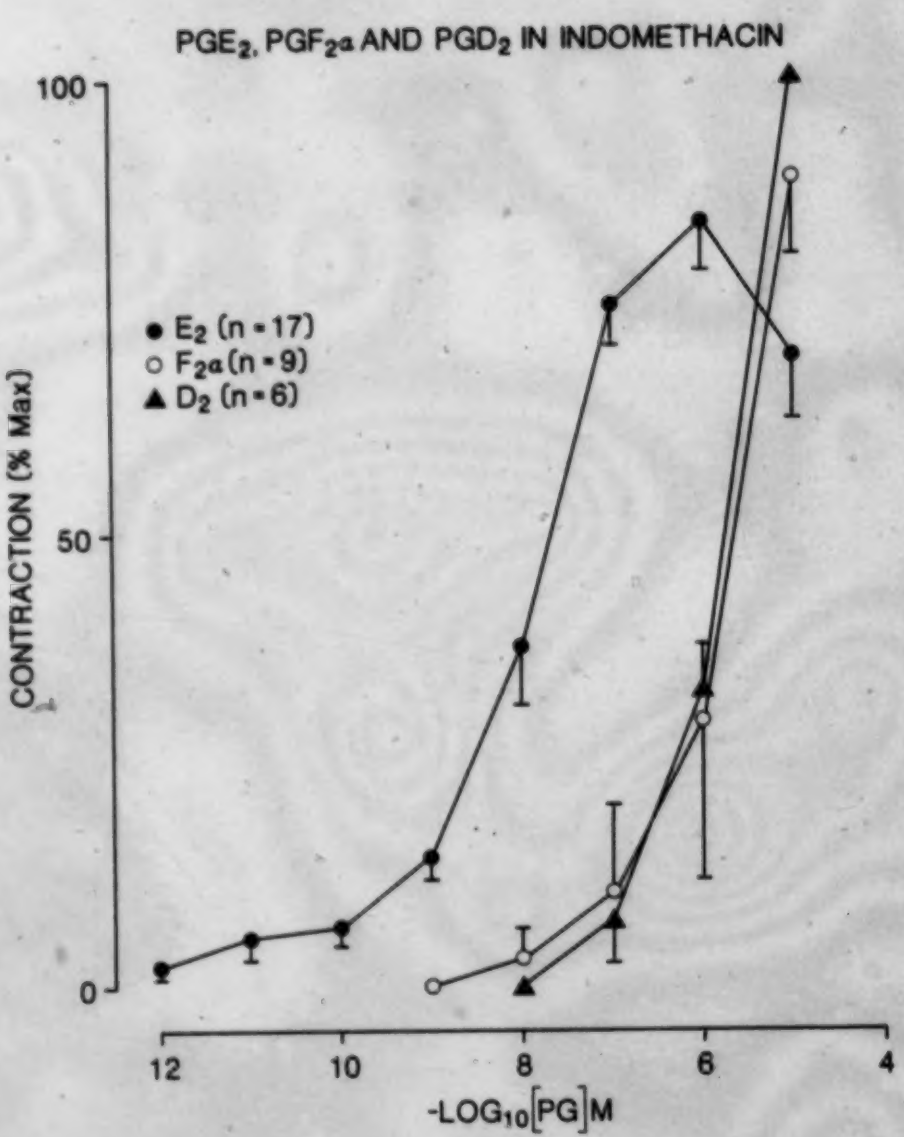

PGE2, PGF2α AND PGD2 IN INDOMETHACIN
100
50
0
CONTRACTION (% Max)
● E2 (n = 17)
○ F2α (n = 9)
▲ D2 (n = 6)
12
10
8
6
4
-LOG10[PG]M

FIGURE 16. Concentration-response curves for PGE_2, $PGF_{2\alpha}$ and PGD_2 in human gallbladders with chronic mild and advanced cholecystitis in the presence of 10^{-5} M indomethacin. Points represent the mean ± SEM for n observations. Results are expressed as a percent of the maximal response to 10^{-3} M acetylcholine.

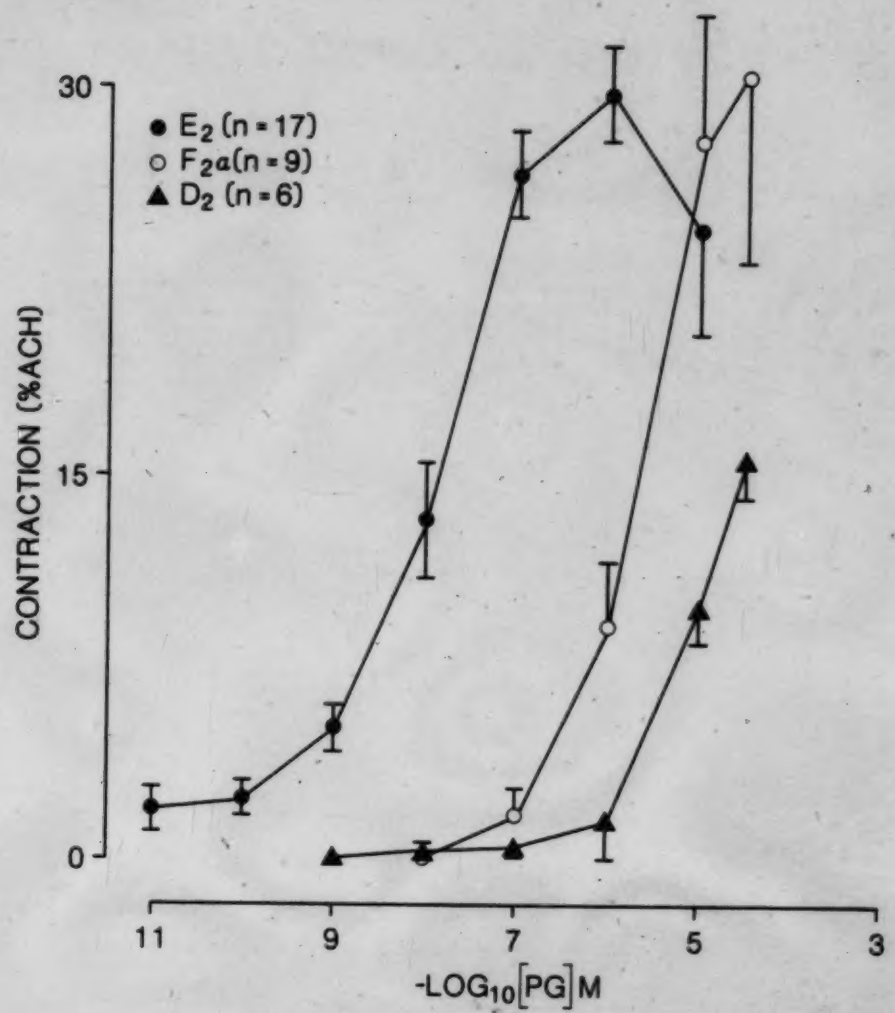
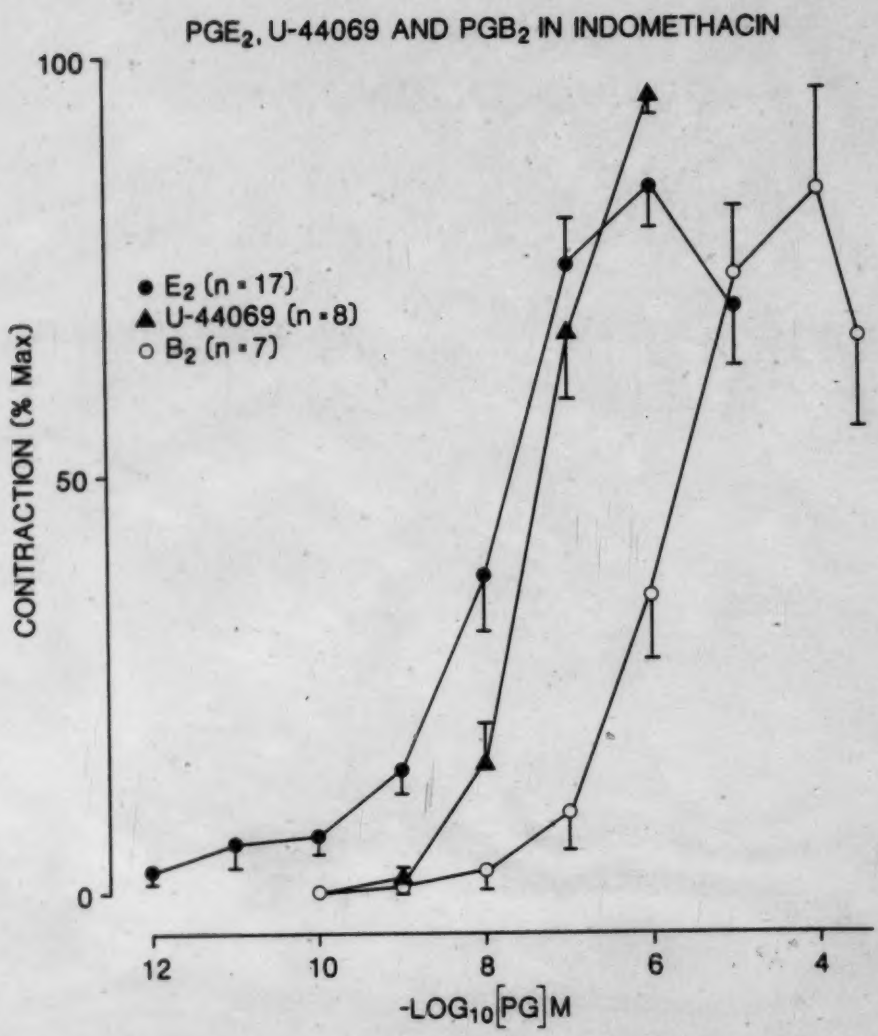

PGE2 , PGF2α AND PGD2 -INDOMETHACIN
30
15
0
CONTRACTION (%ACH)
● E2 (n = 17)
○ F2α(n = 9)
▲ D2 (n = 6)
11
9
7
5
3
-LOG10[PG]M

FIGURE 17. Concentration-response curves to PGE_2, U-44069 and PGB_2 in human gallbladders with chronic mild and advanced cholecystitis in the presence of 10^{-5} M indomethacin. Points represent the mean ± SEM for n observations. Results are expressed as percentages of the maximal response for each agonist.


PGE2, U-44069 AND PGB2 IN INDOMETHACIN
100
50
0
CONTRACTION (% Max)
● E2 (n = 17)
▲ U-44069 (n = 8)
○ B2 (n = 7)
12
10
8
6
4
-LOG10[PG]M

and guinea-pig muscle strips; the presence of indomethacin was not necessary to produce consistent concentration-dependent contractions in guinea-pig gallbladder (Fig. 18). Threshold responses occurred at the same concentration in both human and guinea-pig gallbladder. A maximum response was not obtained at the highest concentration (3×10^{-5} M) tested in each species.

Arachidonic Acid and Prostaglandin A_2

Arachidonate produced no effect on 4 human gallbladders with CMC or CAC in a concentration range from 10^{-11} to 10^{-5} M, and had only weak contractile effects at 10^{-4} M. Similarly, on 3 human gallbladders with CMC, PGA_2 had no effect in a concentration range from 10^{-11} to 10^{-5} M.

Prostacyclin

PGI_2 produced concentration-dependent contractions in guinea-pig but had no effect on 6 human gallbladders with CMC or CAC in a concentration range from 5.7×10^{-11} to 2.9×10^{-6} M (Fig. 19). The threshold response in guinea-pig gallbladder occurred between 10^{-11} and 10^{-10} M and concentration-response curves both in the absence and presence of indomethacin were not significantly different. A maximum response to guinea-pig gallbladder was not obtained at 2.9×10^{-6} M.

Leukotrienes

LTC_4 and LTD_4 produced concentration-dependent contractions in guinea-pig gallbladder but had no effect on 6 human gallbladders with CMC or CAC in a concentration range from 10^{-14} to 3×10^{-7} M (Fig. 20). Similar responses were observed in guinea-pig muscle strips

FIGURE 18. Concentration-response curves for PGD_2 in human gallbladders with chronic mild and advanced cholecystitis in the presence of 10^{-5} M indomethacin and in guinea-pig gallbladders. Points represent the mean ± SEM for n observations. Results are expressed as a percent of the maximal response to 10^{-3} M acetylcholine.

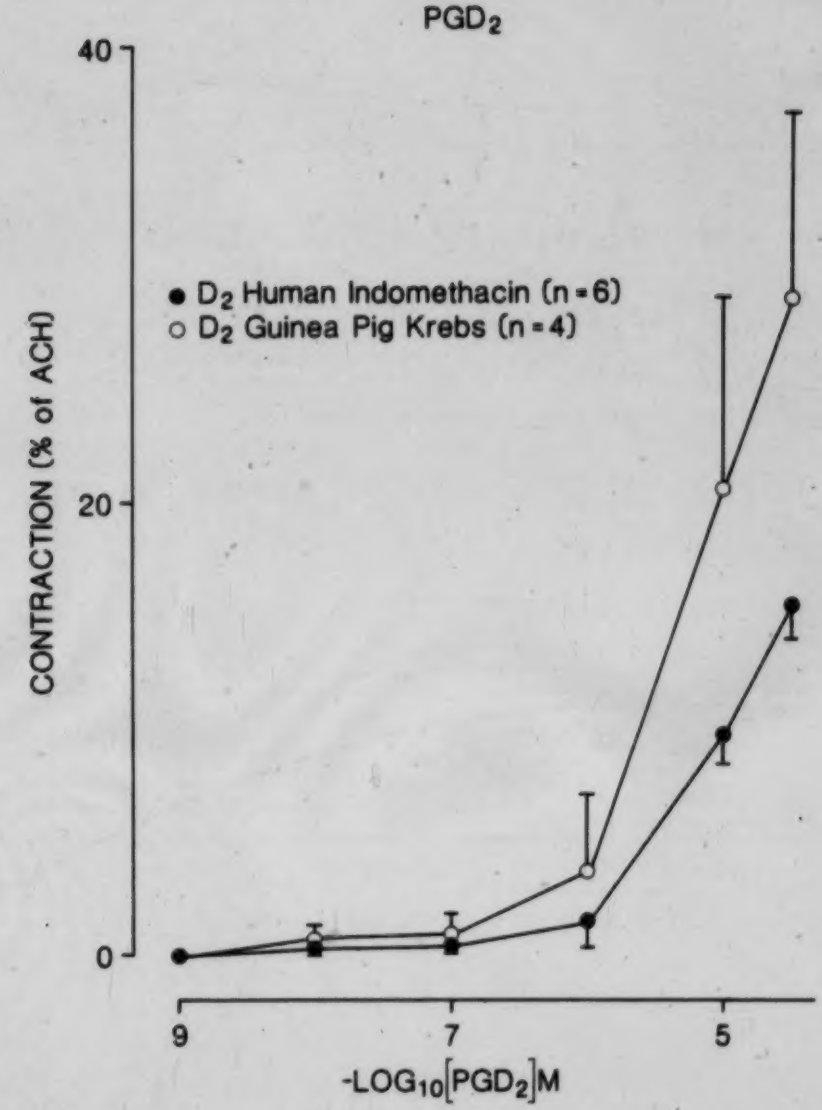

PGD2
40
20
0
CONTRACTION (% of ACH)
● D2 Human Indomethacin (n = 6)
○ D2 Guinea Pig Krebs (n = 4)
9
7
5
-LOG10[PGD2]M

FIGURE 19. Concentration-response curves for prostacyclin in human (chronic mild and advanced cholecystitis) and guinea-pig gallbladders both in the presence and absence of 10^{-5} M indomethacin. Points represent the mean ± SEM for n observations. Results are expressed as a percent of the maximal response to 10^{-3} M acetylcholine.

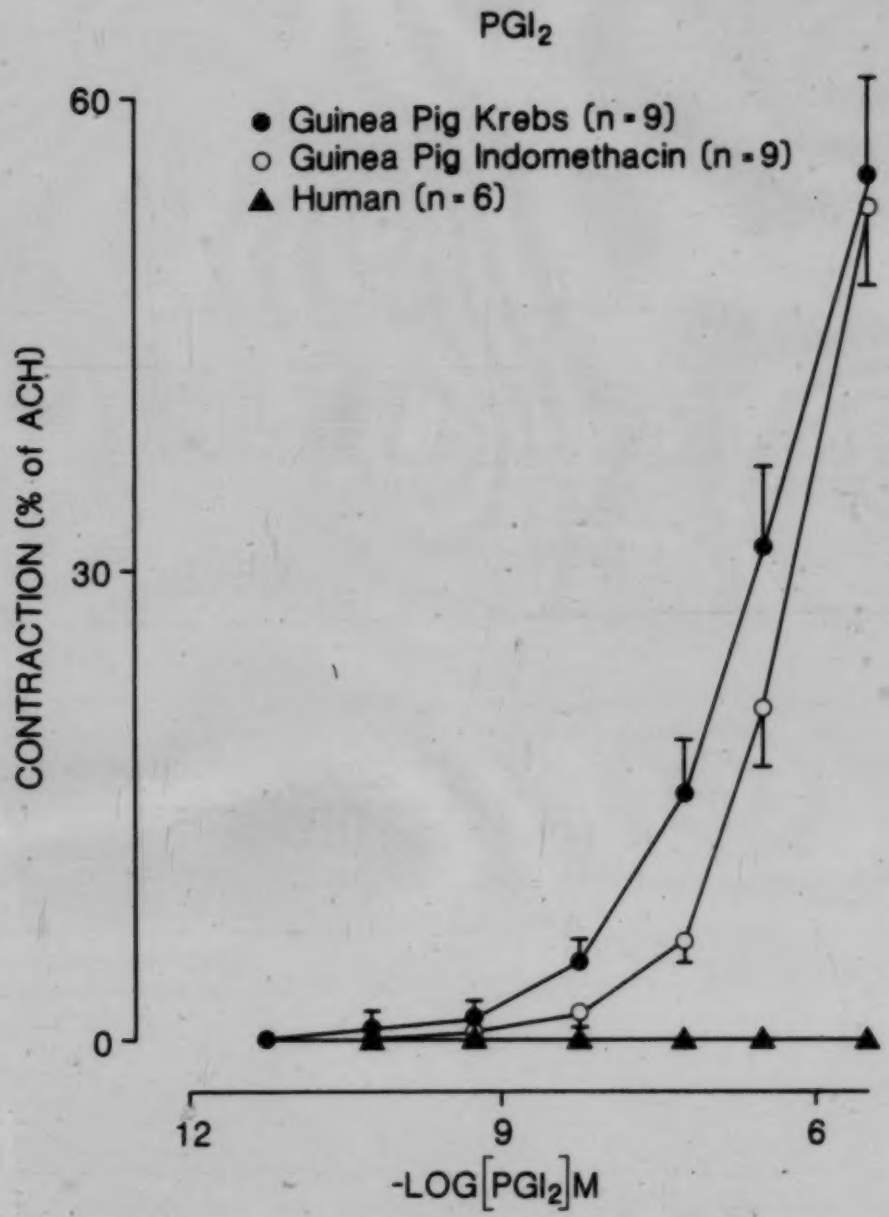
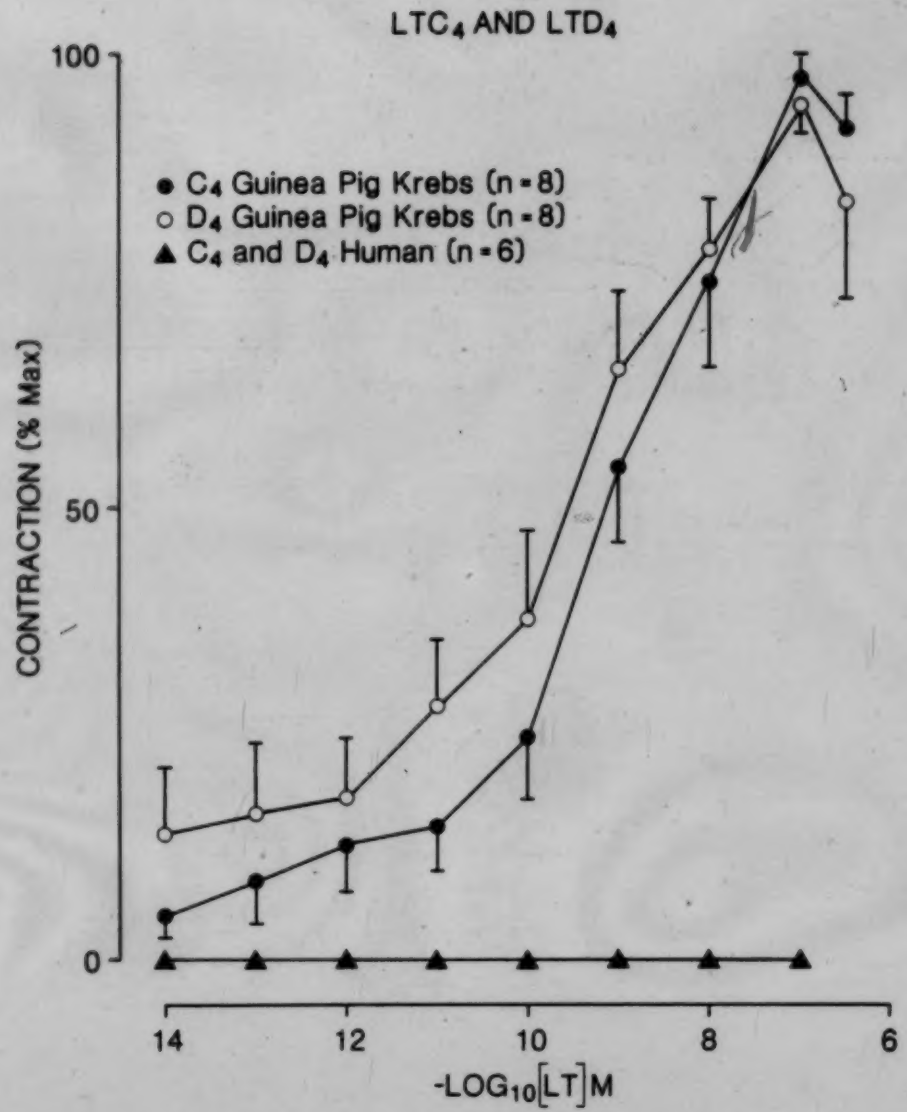

PGI2
60
30
0
CONTRACTION (% of ACH)
● Guinea Pig Krebs (n = 9)
○ Guinea Pig Indomethacin (n = 9)
▲ Human (n = 6)
12
9
6
-LOG[PGI2]M

FIGURE 20. Concentration-response curves for LTC_4 and LTD_4 in human gallbladders with chronic mild and advanced cholecystitis in the presence and absence of 10^{-5} M indomethacin and in guinea-pig gallbladder in the absence of indomethacin. Points represent the mean ± SEM for n observations. Results are expressed as a percentages of the maximal response for each agonist.


LTC4 AND LTD4
100
50
0
CONTRACTION (% Max)
● C4 Guinea Pig Krebs (n = 8)
○ D4 Guinea Pig Krebs (n = 8)
▲ C4 and D4 Human (n = 6)
14
12
10
8
6
-LOG10[LT]M

incubated with indomethacin (10^{-5} M). As seen in Fig. 10, the contractile responses to LTC_4 and LTD_4 are quite similar and very different from responses to PGD_2 and PGI_2 in the guinea-pig gallbladder. Even in the presence of indomethacin, LTC_4 and LTD_4 produced no responses in human gallbladder. The threshold response in guinea-pig gallbladder occurred between 10^{-15} and 10^{-14} M and the maximum response occurred at 10^{-7} M. The ED_{50} (95% confidence limits) for LTC_4 and LTD_4 were 818 pM (466 - 3620) and 409 pM (93 - 1810), respectively; these results are not significantly different ($P > 0.05$). LTB_4 produced no responses in human gallbladder (n = 3) or guinea-pig gallbladder (n = 3), both with and without indomethacin. Addition of the leukotriene antagonist FPL55712 to the bath in a concentration of 1 μg/ml produced partial antagonism of the responses in 3 guinea-pig gallbladders to LTC_4 and LTD_4.

Responses of Gallbladders with Acute Cholecystitis

Motility studies were performed on 13 gallbladders with AC. PGE_1 and PGE_2 in the absence of indomethacin produced either small relaxations or no response; no contractile responses were seen (n = 10 gallbladders). PGE_1 and PGE_2 in the presence of indomethacin produced similar responses, except in 2 gallbladders with little or no fibrosis where concentration-dependent contractions were seen; in the remaining gallbladders, moderate to advanced fibrosis was present.

Responses of Gallbladders with Hydrops

In the 6 gallbladders with hydrops, no contractile responses to PGE_1 and PGE_2 were seen even at concentrations as high as 10^{-5} M.

Occasionally, relaxant responses were seen occurring in those strips containing varying amounts of hypertrophic muscle fibres.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Prostaglandin release from human gallbladder was examined in both the spontaneous and the stimulated states. Spontaneous prostaglandin release from 4 gallbladders with CMC and CAC revealed release of PGE_2 in 3 of these gallbladders. Calcium ionophore-stimulated prostaglandin release from 11 gallbladders from all three disease categories showed that PGD_2 was the predominant prostaglandin released as shown in Table 2. Acetylcholine-stimulated prostaglandin release from 4 gallbladders that were "pooled" revealed small quantities of PGE_2 and $PGF_{2\alpha}$; measurement of prostaglandin release from the same 4 gallbladders that were "pooled" showed no CCK-induced prostaglandin release. Two gallbladders with AC revealed spontaneous production of $PGF_{2\alpha}$ only. Prostaglandin release in both spontaneous and stimulated states was studied only qualitatively, not quantitatively.

Leukotriene release from human gallbladder was similarly studied in the spontaneous and the stimulated states. Spontaneous release from 4 gallbladders with CMC and CAC revealed small amounts of LTB_4 and larger amounts of LTC_4. Calcium-ionophore leukotriene release from 2 gallbladders with CMC and CAC revealed small amounts of LTB_4 and larger amounts of LTC_4. Acetylcholine and cholecystokinin-stimulated leukotriene release revealed LTC_4 and LTD_4, and LTB_4, respectively. Similarly, leukotriene release was studied qualitatively and not quantitatively.

TABLE 2

Calcium Ionophore A23187-Stimulated Prostaglandin Release from the Human Gallbladder (n = 11)

Prostaglandin	%, Mean ± SEM
1. PGD_2	76.70 ± 7.50
2. PGE_2	7.36 ± 2.71
3. $PGF_{2\alpha}$	16.03 ± 7.83

This includes prostaglandin release from 5 gallbladders with CMC, 3 with AC, 2 with CAC and 1 with hydrops

DISCUSSION

The objectives of this study were twofold. First, to assess the relationship between prostaglandins and human gallbladder motility and second, to examine prostaglandin release from the human gallbladder. Gallbladders were obtained from patients undergoing cholecystectomy; since the operation is performed to remove diseased tissue, and only three histologically normal organs were obtained for study, this unfortunately limits the comparison between diseased and normal prostaglandin-mediated gallbladder responses in human tissues.

The results obtained in this study included the use of a histological scoring system that facilitated placement of the gallbladders into various disease categories; this was analyzed to see if a relationship existed between different disease groups and the clinical presentation. Motility effects were studied in regard to spontaneous activity, muscle tension and endogenous tone in the three disease groups. Individual drug-induced motility studies were performed in the chronic mild and chronic advanced cholecystitis groups of gallbladders; it was found that prostaglandins of the E series were the most potent in effecting human gallbladder muscle contraction, whereas prostacyclin and the leukotrienes had no effect on the human gallbladder. Responses in those gallbladders with acute cholecystitis and hydrops were also examined and drug-induced motility effects were found to be quite different. Lastly, prostaglandin release from the human gallbladder was examined with PGE_2 and $PGF_{2\alpha}$ being released spontaneously and PGD_2 being the predominant prostaglandin released following calcium ionophore stimulation.

The preceeding results are all from *in vitro* studies of human gallbladder muscle strips. Clearly *in vivo* studies using either intra-arterial or intraluminal administration of prostaglandins would be ideal to examine gallbladder motility but this raises several issues. First and foremost are ethical considerations because a study such as this would involve invasive techniques. Second, the study would have to take into account the many subtle vascular, hormonal and neural mediated effects on *in vivo* gallbladder motility; moreover, bile flow dynamics regulated by liver production, sphincter of Oddi contraction and the as yet unanswered question of cystic duct motility, may well affect gallbladder responses to exogenous prostaglandins. The last issue involves technical difficulties related to *in vivo* prostaglandin administration.

Studies *in vitro* would naturally circumvent these issues but the critical problem is that the effects of any drug *in vitro* may be different from those *in vivo*. However, prostaglandins are unique in that they are released intracellularly in response to many types of stimuli[51,52,64] and produce very specific local effects; the bloodstream is therefore not necessary for prostaglandin-mediated responses. Thus, exogenous prostaglandin administration with its subsequent diffusion into an isolated tissue strip and production of a response closely simulates prostaglandin effects *in vivo*.

HISTOLOGY

In order to assess the possible differential effect of

prostaglandins in the presence of different grades of cholecystitis, the gallbladders were classified according to a histological scoring system that had first been established by Lennon[133]. All gallbladders were classified as chronic mild, chronic advanced or acute cholecystitis based on the presence or absence of several histological parameters (Fig. 3). As expected, those gallbladders with chronic mild cholecystitis were the least diseased (lowest histological score), in contrast to those with acute cholecystitis that were severely diseased and had the highest histological score (Fig. 4).

The traditional classification of gallbladder histopathology is based on criteria offering only two grades of disease, acute or chronic cholecystitis. The scoring system used here is based on an objective quantification of histopathology which allows for three grades of cholecystitis; more importantly, it provides for a better association between histopathology and motility studies. The overall disease distribution in this study (Fig. 5) with chronic mild cholecystitis gallbladders being the commonest is somewhat different than Lennon's study[133] where he found gallbladders of the chronic advanced cholecystitis type to be the most common. This is probably due to the different groups of patients undergoing cholecystectomy in these two studies.

ASSOCIATION WITH CLINICAL PRESENTATION

The female to male preponderance in this study of 2.6 : 1 is somewhat less than that quoted in the literature[126,133] of 4 : 1. One

might have expected that patients with acute cholecystitis have prominent clinical findings and abnormal laboratory data; but, in general, these clinical parameters had poor correlation in predicting the extent of pathological change in the gallbladder. This is in agreement with Lennon's findings[180].

Motility results were not affected by the presence of gallstone pancreatitis in 6 patients, as all motility studies were in vitro and hence not affected by nearby inflammatory conditions.

MOTILITY EFFECTS

Spontaneous Activity and Muscle Tension

Several smooth muscle preparations when studied in an in vitro fashion (i.e., in the absence of hormonal and neural stimuli) possess spontaneous activity. Spontaneous activity in human gallbladder smooth muscle is due in whole or in part to endogenous prostaglandins because when tissues are incubated with indomethacin, there is a suppression or complete abolition of spontaneous activity.

There is ample evidence in the literature that prostaglandins are pro-inflammatory and are found in increased quantities in many forms of inflammatory responses[58-60]. More specifically, in the gastrointestinal tract, many investigators have found increased levels of prostaglandins in ulcerative colitis[111-114] and Crohn's disease[122,123] - two diseases of the bowel with an inflammatory component which at times can be marked. It is therefore reasonable to assume that those gallbladders with an increased amount of inflammation

- the acute cholecystitis group - contain an increased amount of prostaglandins. That prostaglandins are contained in the human gallbladder has already been demonstrated by Wood and Stamford[178].

The percentage of strips displaying spontaneous activity was greatest in those gallbladders with chronic mild cholecystitis, less so in the acute cholecystitis and least in the chronic advanced cholecystitis groups (Fig. 7A). One would expect those gallbladders with acute cholecystitis to have the highest percentage of spontaneous activity but the presence of fibrosis and the fact that most "acute" gallbladders are usually superimposed on a chronic cholecystitis, are probable reasons why this is not so. However, the percentage of strips demonstrating an effect of indomethacin on spontaneous activity (Fig. 7B) and the percentage of strips demonstrating an effect of indomethacin on tone (Fig. 7C) is least in the acute cholecystitis group, possibly consistent with the theory that this group of gallbladders may be under the influence of a high level of prostaglandins. However, the converse may be true, in that there may be a very low level of prostaglandins in these acutely inflamed tissues.

Endogenous Tone due to Prostaglandins

Nakata et al.[173] found that PGE_1 and PGE_2 in guinea-pig gallbladder are responsible for modulating resting tone and cholinergically-induced contractile responses. In human gallbladders with chronic mild or chronic advanced cholecystitis, PGE_1 and PGE_2 produced inconsistent or no responses in those strips with marked spontaneous activity, whereas all other prostaglandins ($PGF_{2\alpha}$, PGD_2, PGB_2 and U-44069) produced concentration-dependent contractions

regardless of the level of spontaneous activity. Similarly, in those gallbladders with acute cholecystitis, PGE_1 and PGE_2 produced inconsistent responses, whereas $PGF_{2\alpha}$ always produced contractions in the presence of indomethacin. Additionally, in the three histologically normal gallbladders, PGE_2 was the only prostaglandin inducing contractile responses that were not augmented in the presence of indomethacin; this did not occur with PGE_1, $PGF_{2\alpha}$ and U-44069.

These findings indicate that prostaglandins of the E series contribute to spontaneous activity which can possibly be equated to resting tone and also to basal prostaglandin levels. This is further substantiated by the fact that PGE_2 was the only prostaglandin spontaneously released from 3 out of 4 gallbladders with chronic mild and advanced cholecystitis. To verify this, selective prostaglandin antagonists could be used to reduce spontaneous activity and tone and if the "PGE_2 antagonist" was successful in this regard, this would provide direct evidence; however, such selective antagonists are not available at the present time. There are no human studies relating prostaglandins and resting tone in the gallbladder, but the implication that PGE_2 is of importance in endogenous tone is consistent with Nakata's findings in the guinea-pig.

INTERPRETATION OF MOTILITY STUDIES AND PROSTAGLANDIN RELEASE

Human gallbladder motility studies with the various prostaglandins have given consistent results only in the presence of indomethacin. This has been alluded to in the dog gallbladder by Mroczka et al. (176)

who stated that isolated preparations were under the influence of high levels of endogenous prostaglandins and that only following pretreatment with indomethacin could consistent concentration-dependent contractions to prostaglandins be obtained (personal communication). Mroczka et al. and Wood et al. [167] are the only two groups of investigators to examine the effects of prostaglandins on human gallbladder and both studies met with little success in defining a role of prostaglandins in human biliary motility. Hence, a comparison of the findings in this study with other reports in the literature is not possible.

Motility studies with PGE_2 carried out in one gallbladder that contained no gallstones and that was histologically normal, suggest low endogenous prostaglandin levels. Contractile responses to PGE_2 in the absence of indomethacin were of the same magnitude as those in the presence of indomethacin indicating that endogenous prostaglandin levels were low in this "normal" gallbladder; indomethacin was therefore not necessary to suppress endogenous prostaglandins and thereby exogenous prostaglandins were able to effect contractile responses.

Acetylcholine and PGE Responses

Acetylcholine induced contractile responses in all gallbladder strips from all three disease groups. The mean acetylcholine response was similar in strips from chronic mild and chronic advanced cholecystitis gallbladders, whereas those with acute cholecystitis exhibited responses only one-half that of those tissues with milder disease (Table 1). These findings are in accordance with those of Lennon[180].

The smaller responses to acetylcholine in the "acute" gallbladders

may be due to differences in muscle thickness; however, Feeley (unpublished data) has measured the transverse muscle thickness in the three disease groups and found the results to be not significantly different. Another factor might be the amount of fibrosis present in the wall that physically limits the contractile response to any agonist. The amount of fibrosis in gallbladders with chronic advanced cholecystitis and acute cholecystitis on light microscopy was similar, but the response to acetylcholine in the chronic advanced cholecystitis group was even greater than the response in the chronic mild cholecystitis group where fibrosis was minimal (Table 1). This speaks against fibrosis being a significant factor. Other factors include the health of the muscle in acute disease which is very difficult to quantify; the inflammatory cell infiltrate which is sometimes extensive; and red blood cell extravasation, hyperemia and edema. It is conceivable that all the above factors may well affect the contractile (or relaxant) response to an agonist.

Agonists produce a biologic response in a tissue by interacting with a receptor; the magnitude of the response is proportional to the fraction of total receptor sites occupied by the agonist. It is reasonable to assume that these receptors may be damaged in the presence of acute disease or that the agonist may take excess time to reach the receptors (thereby allowing inactivation) and effect a response. Both these hypotheses may account for a smaller response with acetylcholine in the presence of acute disease.

Acetylcholine responses in the "normal" group of gallbladders were approximately half that of the chronic mild cholecystitis group and is probably due to the very narrow bands of muscle seen on light microscopy

in the absence of disease, compared to the muscle thickening seen in the presence of disease.

Cholecystokinin[181], and acetylcholine released via vagal nerve stimulation are two potent contractile agents of normal gallbladder motility. The magnitude of response to a maximally effective concentration of PGE_1 and PGE_2 was less than a third of the response of the same muscle strip to acetylcholine in gallbladders with chronic mild and chronic advanced cholecystitis (Fig. 12), indicating that prostaglandins alone are relatively weak agents in effecting human gallbladder contraction. However, it is clear that human gallbladder muscle does possess prostaglandin receptors.

Prostaglandin E_1 and E_2

PGE_1 and PGE_2 were the most potent prostaglandins in effecting contractile responses in human gallbladder. As mentioned previously, there are no human studies with prostaglandins for comparison; however, Wood *et al.*[167] also found PGE_2 to be a potent agent in effecting guinea-pig gallbladder contraction. Although the threshold responses in this study at 10^{-12} and 10^{-11} molar concentration are *in vitro*, these are very low concentrations and are probably compatible with physiologic *in vivo* effects. The fact that prostaglandins of the E series are the most potent may have some relationship with the finding that PGE_2 was spontaneously released from 3 out of 4 gallbladders with chronic mild and chronic advanced cholecystitis. The results of acetylcholine and cholecystokinin-stimulated prostaglandin release indicate that these two agents are not significantly involved in triggering prostaglandin release and that their contractile activity in human gallbladder is not

likely to be mediated through prostaglandins.

Other Prostaglandins

Calcium ionophore-stimulated prostaglandin release measured by high performance liquid chromatography revealed that human gallbladder is capable of synthesizing all the "classic" prostaglandins (PGE_2, $PGF_{2\alpha}$ and PGD_2) with PGD_2 by far being the predominant prostaglandin released. Calcium ionophore causes an immediate and large increase in intracellular calcium in order to stimulate prostaglandin release; this is not very physiologic and may indeed harm the cell. Therefore, this method of stimulated prostaglandin release may not apply *in vivo* and may be completely misleading. More "physiologic" prostaglandin stimulants (acetylcholine and cholecystokinin) did not show release of PGD_2, again questioning the validity of calcium ionophore. Wood and Stamford[178] similarly found that human gallbladder released these three prostaglandins (PGE_2, $PGF_{2\alpha}$ and PGD_2), but their study did not use high performance liquid chromatography and prostaglandin release was not stimulated. Since PGD_2 is not very potent in effecting human and guinea-pig contractile responses (Fig. 18), it therefore does not appear to play an important role in contractility; however, being the predominant prostaglandin released, PGD_2 may exist in a homeostatic balance with PGE_2 in determining resting tone of the human gallbladder. Alternatively, PGD_2 may be involved in a completely different function; a function that prostaglandins are known to mediate at least in animal gallbladders[160-162,165-167], namely that of mucosal secretion.

Prostacyclin

Prostacyclin has been found in large quantities in the rat[182] and bovine[183] stomach, and is the major arachidonate metabolite in the canine gastrointestinal tract[184]. There are no studies confirming PGI_2 levels in human gallbladder but Booker & LaMorte [177] have found the breakdown product of PGI_2 - 6-keto-$PGF_{1\alpha}$ - in guinea-pig gallbladder. The present study found PGI_2 to be a relatively weak agent in contracting guinea-pig gallbladder and to have no effect on human gallbladder motility (Fig. 19). Robert[103] has found PGI_2 to be anti-enteropooling (inhibit intraluminal secretion) in rats and possibly PGI_2 has a role in fluid transport in the human gallbladder.

Leukotrienes

Lipoxygenase products are involved in the inflammatory response and are potent mediators of immediate hypersensitivity. This study demonstrated release of LTB_4 and larger amounts of LTC_4 from human gallbladder (both spontaneously and stimulated by calcium ionophore). That LTD_4 was not found is not surprising as it is synthesized from LTC_4. The leukotrienes had no effect on human gallbladder motility, whereas LTC_4 and LTD_4 were very potent inducers of guinea-pig gallbladder contraction (Fig. 20). These contractile effects occurred in the presence of indomethacin, indicating they were not mediated through the generation of cyclooxygenase products and probably also indicates the existance of separate leukotriene receptors. These findings in the guinea-pig gallbladder are almost identical to those found by Yusko *et al.*[185] Thus, leukotrienes do not appear to have a role in human gallbladder motility but being present in human tissue,

possess some as yet unknown function.

Gallbladders with Acute Cholecystitis

The initiating event in acute cholecystitis is gallbladder outlet obstruction secondary to a stone impacted in the gallbladder neck. This has been confirmed radiologically in the literature by intravenous cholangiography and hepaticoiminodiacetic acid (HIDA) scanning which demonstrates non-filling of the gallbladder secondary to cystic duct obstruction by a calculus. The fact that only one of the fifteen gallbladders with acute cholecystitis was found to have a stone impacted in the cystic duct intraoperatively, is probably due to stone dislodgement during the interval between hospital admission and operative intervention. Csendes and Sepulveda[186] demonstrated that patients with acute cholecystitis have a significant increase in gallbladder intraluminal pressure, over 3 times that of patients with chronic cholecystitis. This leads to the classic "tense viscus" often palpated preoperatively and sometimes seen intraoperatively; since it is known that mechanical stimuli (e.g., stretch)[64] cause prostaglandin release, this provides another reason for suspecting elevated prostaglandin levels in those gallbladders with acute cholecystitis.

In general, as the severity of inflammation progressed throughout the three disease categories (i.e., from chronic mild to chronic advanced to acute cholecystitis), the percentage of tissue strips demonstrating concentration-dependent contractions to PGE_1 and PGE_2 decreased. In other words, in the absence of indomethacin, those strips from gallbladders with chronic mild cholecystitis showed the geatest percentage of response, in contrast to those from gallbladders with

acute cholecystitis where no responses were induced by PGE_1 and PGE_2. Additionally, in the presence of indomethacin, which suppresses endogenous prostaglandin release, concentration-dependent contractions were always seen in muscle strips from gallbladders with chronic mild and chronic advanced cholecystitis; however, in the acute cholecystitis group, only 2 out of 13 gallbladders demonstrated concentration-dependent contractions. It is reasonable to state that in the chronic mild and chronic advanced cholecystitis gallbladders, indomethacin sufficiently suppressed endogenous prostaglandin levels and "permitted" exogenous prostaglandins to produce contractile responses. In the acute cholecystitis gallbladders, where prostaglandin production is assumed to be higher, contractile responses in the presence of indomethacin only rarely occurred. This again provides further evidence for the hypothesis that this group of gallbladders possibly contain high levels of endogenous prostaglandins.

Conversely, one could argue that prostaglandin production in these "acute" gallbladders could be very low, possibly secondary to the severe inflammatory process with intracellular damage and impairment of prostaglandin synthesis. If this were the case, one would also not expect exogenous prostaglandins (with or without indomethacin) to effect a response, as there probably needs to be a critical level of prostaglandins present in the tissue (both endogenous and exogenous) for a response to occur. However, in light of all the previous evidence, I feel that those gallbladders with acute disease are continuously being stimulated to produce high levels of prostaglandins. Quantitative prostaglandin release by high performance liquid chromatography will probably provide the final answer.

There could also be a different spectrum of prostaglandins produced. However, this did not occur with calcium ionophore-stimulated prostaglandin release, but in two "acute" gallbladders examined for spontaneous release, $PGF_{2\alpha}$ was the only prostaglandin released. Although the number of gallbladders examined is small, a different spectrum of prostaglandins may contribute to the altered motility studies observed in gallbladders with acute disease.

Responses of Gallbladders with Hydrops

These are gallbladders distended with mucus due to an impacted stone and therefore have a high intraluminal pressure for varying periods of time. Marked fibrosis is the predominant feature. Acetylcholine, and PGE_1 and PGE_2 responses in this group were very small (Table 1, Fig. 13) and probably accounts for the lack of response in this group of gallbladders. More importantly, as Dumont et al.(170) have alluded to, prostaglandins may be involved in inducing intraluminal secretion in gallbladders with hydrops.

CONCLUSION

The pathophysiology of gallstones is complex but mucous production appears to be a critical step in gallstone development. Mucous production and prostaglandins are intimately related; thus, gallstone pathogenesis may be related to prostaglandins. Acute cholecystitis is mediated by the potent cytotoxic agent, lysolecithin. Its actions in the gallbladder are abolished by indomethacin indicating prostaglandin potentiation of cholecystitis. In animal studies, prostaglandins induce intraluminal fluid secretion and gallbladder contraction, and this study has determined that prostaglandins are potent contractile agents in the human gallbladder.

More specifically, this study found that

1. PGE_1 and PGE_2 are potent spasmogens of human gallbladder in vitro.
2. PGE_2 may be involved in regulation of spontaneous activity and endogenous tone in human gallbladder. PGE_2 is also the main prostaglandin spontaneously released from human tissue.
3. Increased levels of prostaglandins may be present in those gallbladders with acute cholecystitis.
4. Prostacyclin has no effect on human gallbladder motility.
5. Leukotrienes are released from human tissue but have no effect on human gallbladder motility. However, leukotrienes are very potent spasmogens of guinea-pig gallbladder.
6. The predominant prostaglandin released from human gallbladder by calcium ionophore-stimulated prostaglandin release is PGD_2.

7. The role of prostaglandins in human intraluminal mucosal secretion is yet to be determined.

These findings, in addition to the fact that indomethacin is an effective agent in relieving biliary pain, strongly implicates prostaglandins in mediating the pain of acute cholecystitis, fluid secretion and gallbladder contraction.

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